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L1 8818504 METHOD

=> s l1 and fluorescence energy transfer

L2 898 L1 AND FLUORESCENCE ENERGY TRANSFER

=> s l2 and FRET

L3 62 L2 AND FRET

=> s l3 and phOX

L4 0 L3 AND PHOX

=> s l2 and probe

L5 162 L2 AND PROBE

=> s l5 and biodipyl

L6 0 L5 AND BIODIPYL

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L7 13 L3 AND ASSAY

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L8 11 DUP REMOVE L7 (2 DUPLICATES REMOVED)

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L8 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2001 ACS

2000:881363 Document No. 134:39156 **Fluorescence energy transfer** probes with stabilized conformations. Coook, Ronald M. (Biosearch Technologies, Inc., USA). PCT Int. Appl. WO 2000075378 A1 20001214, 71 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US16148 20000608. PRIORITY: US 1999-PV138376 19990609.

AB The present invention provides a class of Conformationally Assisted Probes

(CAPs) comprising (a) a nucleic acid moiety; (b) an energy donor moiety; (c) an energy acceptor moiety; and (d) one or more stabilizing moieties. Stabilizing groups are: satd./unsatd. hydrocarbons, steroids, fatty acids, fatty alcs. etc., e.g. cholesterol, polyethylene glycol. Typical fluorophores are: fluorescein and TAMRA. The CAP probes are useful as detection agents in a variety of DNA amplification/quantification strategies, including 5'-nuclease **assay** (PCR-Taqman), Strand Displacement Amplification (SDA) and Nucleic Acid Sequence-Based Amplification (NASBA), Rolling Circle Amplification (RCA), as well as for direct detection of targets in soln. phase or solid phase (e.g. array) **assays**.

L8 ANSWER 2 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS
2000:305549 Document No.: PREV200000305549. Dynamics of NF kappaB and IkappaBalpha studied with green fluorescent protein (GFP) fusion proteins:

Investigation of GFP-p65 binding to DNA by fluorescence resonance energy transfer. Schmid, Johannes A.; Birbach, Andreas; Hofer-Warbinek, Renate; Pengg, Margarete; Burner, Ursul; Furtmueller, Paul G.; Binder, Bernd R.; de Martin, Rainer. Journal of Biological Chemistry, (June 2, 2000) Vol. 275, No. 22, pp. 17035-17042. print. ISSN: 0021-9258. Language: English. Summary Language: English.

AB We investigated the dynamics of nuclear transcription factor kappaB (NF-kappaB) by using fusion proteins of the p65 subunit with mutants of green fluorescent protein (GFP). GFP-NF-kappaB chimeras were functional both in vitro and in vivo, as demonstrated by electrophoretic mobility shift **assays** and reporter gene studies. GFP-p65 was regulated by IkappaBalpha similar to wild type p65 and associated with its inhibitor even if both proteins were linked to a GFP protein. This finding was also verified by fluorescence resonance energy transfer (**FRET**) microscopy and studies showing mutual regulation of the intracellular localization of both GFP chimeras. Incubation of GFP-p65 with fluorescently labeled NF-kappaB-binding oligonucleotides also resulted in **FRET**. This effect was DNA sequence-specific and exhibited saturation characteristics. Application of stopped-flow fluorometry to measure the kinetics of **FRET** between GFP-p65 and oligonucleotides revealed a fast increase of acceptor fluorescence with a plateau after about 10 ms. The observed initial binding rate showed a temperature-dependent linear correlation with the oligonucleotide concentration. The association constant calculated according to

pre-steady state kinetics was $3 \times 10^6 \text{ M}^{-1}$, although equilibrium binding studies implied significantly higher values. This observation suggests that the binding process involves a rapid association with a rather high off-rate followed by a conformational change resulting in an increase of the association constant.

L8 ANSWER 3 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS
2000:439111 Document No.: PREV200000439111. A fluorescence-quenched chitopentaose for the study of endo-chitinases and chitobiosidases. Cottaz, Sylvain (1); Brasme, Bernard; Driguez, Hugues. (1) CERMAV-CNRS, 38041, Grenoble Cedex 9 France. European Journal of Biochemistry, (September, 2000) Vol. 267, No. 17, pp. 5593-5600. print. ISSN: 0014-2956.

Language: English. Summary Language: English.

AB A new fluorogenic substrate displaying intramolecular **fluorescence energy transfer (FRET)** has been synthesized

from NI, NII, NIII, NIV-tetra-acetyl-chitopentaose. Two molecules, a fluorophore (5-(2-aminoethyl) amino-1-naphtalene-sulfonic acid; EDANS) and

a quenching group (dimethylaminophenylazophenyl; DAB) were chemically introduced on to the chitopentaose, one at each end. Among eight enzymes tested, only endo-chitinase and chitobiosidase activities could be specifically assayed by monitoring the variation of fluorescence after enzymatic hydrolysis of this substrate. Chitobioses and N-acetyl-beta-glucosaminidases are not active on the compound, the presence of a bulky chromogenic group at the 2 position of the nonreducing

end of the substrate preventing the binding and thus hydrolysis by these two exo-enzymes. The observation that chitobiosidases are able to hydrolyse a chitooligosaccharide functionalized on both extremities demonstrates the possibility of an endo-action for this class of chitinases, which are generally classified as exo-enzymes. This fluorogenic chitooligosaccharide should prove to be very useful for the detection and the convenient **assay** of chitinolytic activities at nanomolar concentrations.

L8 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS

2001:23837 Document No.: PREV200100023837. **Fluorescence**

energy transfer analysis of DNA structures containing

several bulges and their interaction with CAP. Stuhmeier, Frank;

Hillisch,

Alexander; Clegg, Robert M. (1); Diekmann, Stephan. (1) Department of Physics and Laboratory of Fluorescence Dynamics, Loomis Laboratory of Physics, 1110 West Green Street, Urbana, IL, 61801-3080: rclegg@uiuc.edu USA. Journal of Molecular Biology, (6 October, 2000) Vol. 302, No. 5, pp. 1081-1100. print. ISSN: 0022-2836. Language: English. Summary Language: English.

AB DNA molecules with three bulges separated by double-stranded helical sections of B-DNA were constructed to be used as substrates for DNA-protein binding **assays**. Fluorescence resonance energy transfer (**FRET**) between dye molecules attached to the 5'-ends of the DNA molecules is used to monitor the protein binding. The A5 bulge, which consists of five unpaired adenine nucleotides, alters the direction of the helical axis by approximately 80 to 90degree at every bulge site. Computer molecular modeling facilitated a pre-selection of suitable helix lengths that bring the labeled ends of the three-bulge DNA molecules (60 to 70 base-pairs long) into close proximity. The **FRET** experiments verified that the labeled ends of the helices of these long molecules were indeed close. A series of **FRET** experiments was carried out with two A5 and two A7 bulge molecules. The relative

positions

of the bulges were varied along the central helical DNA sequence (between the bulges) in order to determine the relative angular juxtapositions of the outlying helical arms flanking the central helical region. The global structural features of the DNA molecules are manifested in the **FRET** data. The **FRET** experiments, especially those of the two-bulge series, could be interpreted remarkably well with molecular models based on the NMR structure of the A5 bulge. These models assume that the DNA molecules do not undergo large torsional conformational fluctuations at the bulge sites. The magnitude of the **FRET** efficiency attests to a relatively rigid structure for many of the long 5'-end-labeled molecules. The changes in the **FRET** efficiency of three-bulge structures containing the specific binding sequence of the catabolite activator protein (CAP) demonstrated significant deformation

of

the DNA upon binding of CAP. No direct interaction of CAP with the dyes was observed.

L8 ANSWER 5 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS

2000:368963 Document No.: PREV200000368963. Red laser-induced

fluorescence energy transfer in an immunosystem. Oswald, Bernhard; Lehmann, Frank (1); Simon, Lydia; Terpetschnig, Ewald; Wolfbeis, Otto S.. (1) Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, 93040, Regensburg Germany. Analytical Biochemistry, (May 1, 2000) Vol. 280, No. 2, pp. 272-277. print. ISSN: 0003-2697. Language: English. Summary Language: English.

AB We describe two near-infrared fluorescent squaraine dyes (Sq635 and Sq660), their spectra, their covalent linkage to proteins, and their use as donor and acceptor, respectively, in a fluorescence resonance energy transfer (**FRET**) immunoassay based on the use of red lasers. The dyes show quantum yields of around 10% in the free form and up to 68%

when

bound to proteins. If converted into their N-hydroxysuccinimide esters, they can be linked to free amino groups of proteins. To improve water solubility, two sulfo groups were introduced. The emission spectrum of Sq635 overlaps the absorption spectrum of Sq660, a fact that makes them a useful pair of dyes for use in **FRET** immunoassay which is demonstrated for human serum albumin/anti-human serum albumin.

L8 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS

2000:184868 Document No.: PREV200000184868. PKC membrane association and dissociation kinetics studied by **fret** in living PC12 cell.

Janoshazi, Agnes (1); Geeraert, Virginie; Dupont, Jean-Luc; de Barry, Jean. (1) CNRS, Strasbourg, Cell Neurobiology Laboratory, University

Louis

Pasteur, 4, Blaise Pascal, BAS-RHIN, Strasbourg, 67000 France.

Biophysical

Journal, (Jan., 2000) Vol. 78, No. 1 Part 2, pp. 248A. Meeting Info.:

44th

Annual Meeting of the Biophysical Society. New Orleans, Louisiana, USA February 12-16, 2000 ISSN: 0006-3495. Language: English. Summary

Language:

English.

L8 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2001 ACS

1999:673404 Document No. 131:348227 Fluorescence lifetime imaging of receptor tyrosine kinase activity in cells. Wouters, Fred S.; Bastiaens, Philippe I. H. (Cell Biophysics Laboratory, Imperial Cancer Research

Fund,

London, WC2A 3PX, UK). Curr. Biol., 9(19), 1127-1130 (English) 1999.

CODEN: CUBLE2. ISSN: 0960-9822. Publisher: Current Biology

Publications.

AB We report a highly specific fluorescence lifetime imaging microscopy (FLIM) **method** for monitoring epidermal growth factor receptor (EGFR) phosphorylation in cells based on fluorescence resonance energy transfer (**FRET**). EGFR phosphorylation was monitored using a green fluorescent protein (GFP)-tagged EGFR and Cy3-conjugated anti-phosphotyrosine antibodies. In this **FRET**-based imaging **method**, the information about phosphorylation is contained only in the (donor) GFP fluorescence lifetime and is independent of the antibody-derived (acceptor) fluorescence signal. A pixel-by-pixel ref. lifetime of the donor GFP in the absence of **FRET** was acquired

from the same cell after photobleaching of the acceptor. We show that this calibration, by acceptor photobleaching, works for the GFP-Cy3 donor-acceptor pair and allows the full quantitation of **FRET** efficiencies, and therefore the degree of exposed phosphotyrosines, at each pixel. The hallmark of EGFR stimulation is receptor dimerization and concomitant activation of its intracellular tyrosine kinase domain. Trans-autophosphorylation of the receptor on specific tyrosine residues couples the activated dimer to the intracellular signal transduction machinery as these phosphorylated residues serve as docking sites for adaptor and effector mols. contg. Src homol. 2 (SH2) and phosphotyrosine-binding (PTB) domains. The time-course and extent of EGFR phosphorylation are therefore important determinants of the underlying pathway and resulting cellular response. Our results strongly suggest that secondary proteins are recruited by activated receptors in endosomes, indicating that these are active compartments in signal transduction.

L8 ANSWER 8 OF 11 MEDLINE DUPLICATE 1
 1998186848 Document Number: 98186848. PubMed ID: 9518501. Detection of programmed cell death using **fluorescence energy transfer**. Xu X; Gerard A L; Huang B C; Anderson D C; Payan D G; Luo Y. (Rigel, Inc., 772 Lucerne Drive, Sunnyvale, CA 94086, USA.) NUCLEIC ACIDS RESEARCH, (1998 Apr 15) 26 (8) 2034-5. Journal code: O8L; 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom.

Language:

English.

AB **Fluorescence energy transfer (FRET)**
) can be generated when green fluorescent protein (GFP) and blue fluorescent protein (BFP) are covalently linked together by a short peptide. Cleavage of this linkage by protease completely eliminates **FRET** effect. Caspase-3 (CPP32) is an important cellular protease activated during programmed cell death. An 18 amino acid peptide containing CPP32 recognition sequence, DEVD, was used to link GFP and BFP together. CPP32 activation can be monitored by **FRET assay** during the apoptosis process.

L8 ANSWER 9 OF 11 MEDLINE DUPLICATE 2
 96170587 Document Number: 96170587. PubMed ID: 8600827.
Fluorescence energy transfer immunoassay based on a long-lifetime luminescent metal-ligand complex. Youn H J; Terpetschnig E; Szmazinski H; Lakowicz J R. (Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore 21201, USA.) ANALYTICAL BIOCHEMISTRY, (1995 Nov 20) 232 (1) 24-30. Journal code: 4NK; 0370535. ISSN: 0003-2697. Pub. country: United States.
 Language: English.

AB We describe an immunoassay based on fluorescence resonance energy transfer

(**FRET**). The antigen was human serum albumin (HSA), which was labeled with a ruthenium-ligand complex, [Ru(bpy)2(phen-ITC)]2+. The antibody (IgG) to HSA was labeled with a nonfluorescent absorber,

Reactive

Blue 4. Association of the Ru-labeled HSA with the antibody was detected by three spectral parameters, a decreased quantum yield of Ru-HSA, a decrease in its fluorescence lifetime, and an increase in its

fluorescence

anisotropy. The steady-state anisotropy of Ru-HSA increased approximately

eightfold upon binding to the antibody. These spectral effects were observed both in the direct association of the Ru-HSA with Reactive Blue 4-labeled antibody, and in a competitive **assay** format wherein unlabeled HSA competed with Ru-HSA for the binding sites on the antibody. Some nonspecific interactions of HSA may have occurred with Reactive Blue 4-labeled AHA, a difficulty which can be avoided with a different acceptor. The use of **FRET** provides a reliable means to alter the spectral properties upon antigen-antibody binding. The advantages of a ruthenium-ligand fluorophore include its long-wavelength absorption and emission, long fluorescence lifetime, and high photo-stability. Long wavelengths minimize problems of autofluorescence from biological samples, and long life-times allow off-gating of the prompt autofluorescence.

L8 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2001 ACS

1995:350854 Document No. 122:127536 **Fluorescence energy**

transfer substrates for protease determination. Garman, Andrew John (Zeneca Ltd., UK). PCT Int. Appl. WO 9428166 A1 19941208, 22 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK,

ES,

FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-GB1153 19940527. PRIORITY: GB 1993-10978 19930527.

AB A **method** for the prepn. of a fluorescence resonance energy transfer (**FRET**) substrate having donor and acceptor species on opposite sides of a proteolytic cleavage site and wherein the donor and/or

acceptor species are attached via the side chain(s) of amino acid(s) therein is described. The **method** comprises contacting a reactive donor or acceptor species with a polypeptide substrate having

the

side chain(s) of amino acid(s) therein adapted for reaction with the reactive species and then contacting the substrate so obtained with a corresponding reactive donor or acceptor species. Novel **FRET** substrates so prepd. and their use in **assays** to identify modulators of protease activity are claimed. Substrates of endothelin converting enzyme (ECE) and of staphylococcal V8 protease were modified

as

described and assayed for substrate activity and usefulness. Thus, the amino terminus of human 16-37-[Cys-26]big endothelin-1 was reacted with 5-maleimidofluorescein then with tetramethylrhodamine isothiocyanate to prepd. the **FRET** substrate for ECE.

L8 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS

1995:68838 Document No.: PREV199598083138. Single-turnover kinetics of helicase-catalyzed DNA unwinding monitored continuously by

fluorescence energy transfer. Bjornson, Keith

P.; Amaratunga, Mohan; Moore, Keith J. M.; Lohman, Timothy M. (1). (1) Dep. Biochem., Mol. Biophysics, Washington University Sch. Med., Box

8231,

660 S. Euclid Ave., St. Louis, MO 63110 USA. Biochemistry, (1994) Vol.

33,

No. 47, pp. 14306-14316. ISSN: 0006-2960. Language: English.

AB We describe a fluorescence **assay** that can be used to monitor helicase-catalyzed unwinding of duplex DNA continuously in real time. The **assay** is based on the observation that fluorescence resonance

energy transfer (**FRET**) occurs between donor (fluorescein) and acceptor (hexachlorofluorescein) fluorophores that are in close proximity due to their covalent attachment to the 3' and 5' ends of the complementary strands of a duplex oligodeoxynucleotide. **FRET** results in a reduction in the fluorescence emission intensity of fluorescein in the duplex DNA substrate relative to that observed for fluorescein-labeled single stranded DNA. Therefore, an enhancement of fluorescein fluorescence ($\lambda_{\text{ex}} = 492 \text{ nm}$; $\lambda_{\text{em}} = 520 \text{ nm}$) occurs upon helicase-catalyzed unwinding of the duplex DNA and separation of the complementary strands. The fluorescence **assay** is extremely sensitive, allowing DNA unwinding reactions to be monitored continuously at DNA concentrations as low as 1 nM in a fluorescence stopped-flow experiment. We demonstrate the use of this DNA substrate in pre-steady state, single turnover studies of duplex DNA unwinding catalyzed by the *Escherichia coli* Rep helicase, monitored by fluorescence stopped flow. We show that the fluorescence enhancement monitors Rep-catalyzed DNA unwinding by comparisons with identical kinetic studies carried out using rapid chemical quench-flow techniques. Single turnover kinetic studies performed at 1 nM DNA as a function of excess Rep concentration show that Rep-catalyzed unwinding of an 18 base pair duplex containing a 3'-ss-(dT)-20 tail is biphasic and can be described by the sum of two exponential terms. The observed rate constant of the first phase is independent of (Rep) (20-300 nM) and measures the rapid single turnover unwinding of the duplex DNA by Rep dimers bound in productive complexes ($1.3 \pm 0.2 \text{ s}^{-1}$; $23 \pm 3 \text{ base pairs s}^{-1}$ at 25.0°C). The observed rate constant for the second phase increases linearly with (Rep), reflecting DNA unwinding that is limited by a Rep binding event occurring with a bimolecular rate constant of $(1.8 \pm 0.1) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, which may reflect the rate constant for Rep dimerization on DNA. Kinetic competition studies indicate that both Rep subunits are bound stably to the DNA substrate in the productive complex that is unwound in the fast phase. The results of these kinetic studies are consistent with an active, rolling mechanism for Rep-catalyzed unwinding of DNA (Wong, I., & Lohman, T. M., (1992) *Science* 256, 350). This fluorescence **assay** should greatly facilitate further mechanistic studies of helicase-catalyzed DNA unwinding.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 18:10:32 ON 14 MAY 2001

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L1      8818504 S METHOD
L2      898 S L1 AND FLUORESCENCE ENERGY TRANSFER
L3      62 S L2 AND FRET
L4      0 S L3 AND PHOX
L5      162 S L2 AND PROBE
L6      0 S L5 AND BIODIPYL
L7      13 S L3 AND ASSAY
L8      11 DUP REMOVE L7 (2 DUPLICATES REMOVED)
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=> s 13 and PhOx-Bodipy Fl

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L9      0 L3 AND PHOX-BODIPY FL
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PROCESSING COMPLETED FOR L3

L10 39 DUP REMOVE L3 (23 DUPLICATES REMOVED)

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L10 ANSWER 1 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

2001:163063 Document No.: PREV200100163063. **FRET** analysis indicates that the two ATPase active sites of the P-glycoprotein multidrug transporter are closely associated. Qu, Qin; Sharom, Frances J. (1). (1) Department of Chemistry and Biochemistry, University of Guelph, Guelph, ON, N1G 2W1: sharom@chembio.uoguelph.ca Canada. Biochemistry, (February

6,

2001) Vol. 40, No. 5, pp. 1413-1422. print. ISSN: 0006-2960. Language: English. Summary Language: English.

AB Members of the ABC superfamily carry out the transport of various molecules and ions across cellular membranes, powered by ATP hydrolysis. Substantial evidence indicates that the two catalytic sites of the nucleotide binding domains function in a highly cooperative, alternating sites mode, which suggests the possibility that they interact with each other physically. In this study, **fluorescence energy transfer** experiments were used to estimate the distance between two fluors, each covalently linked to a highly conserved Cys residue (Cys428 and Cys1071) within the Walker A motif of the catalytic site. The vanadatecntdotADPcntdotMg2+ complex was trapped in one catalytic site of membrane-bound or highly purified P-glycoprotein, and the other site was labeled with MIANS (2-(4'-maleimidylanilino)naphthalene-6-sulfonic acid). Following loss of the trapped vanadate complex, the newly vacant site was then labeled with NBD-Cl (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole). The fluorescence properties of the singly labeled P-glycoproteins showed that no energy transfer occurred between MIANS (the donor) and NBD (the acceptor) when they were simply mixed together. On the other hand, the fluorescence emission of the MIANS group in doubly labeled P-glycoprotein was highly quenched as a result of energy transfer to NBD, leading to an estimate of a donor-acceptor separation distance of apprxl6 ANG for P-glycoprotein labeled in the native plasma membrane and apprx22 ANG for P-glycoprotein labeled in detergent solution. The separation of the two fluorophores is compatible with the recently reported crystal structure

of

the Rad50cd dimer, but not with that of the HisP dimer. These results suggest that the two catalytic sites of the P-glycoprotein nucleotide binding domains are relatively close together, which would facilitate cooperation between them during the catalytic cycle.

L10 ANSWER 2 OF 39 CAPLUS COPYRIGHT 2001 ACS

2000:881363 Document No. 134:39156 **Fluorescence energy**

transfer probes with stabilized conformations. Coook, Ronald M. (Biosearch Technologies, Inc., USA). PCT Int. Appl. WO 2000075378 A1 20001214, 71 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE,

NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US16148 20000608. PRIORITY: US 1999-PV138376 19990609.

AB The present invention provides a class of Conformationally Assisted Probes

(CAPs) comprising (a) a nucleic acid moiety; (b) an energy donor moiety; (c) an energy acceptor moiety; and (d) one or more stabilizing moieties. Stabilizing groups are: satd./unsatd. hydrocarbons, steroids, fatty

acids,

fatty alcs. etc., e.g. cholesterol, polyethylene glycol. Typical fluorophores are: fluorescein and TAMRA. The CAP probes are useful as detection agents in a variety of DNA amplification/quantification strategies, including 5'-nuclease assay (PCR-Taqman), Strand Displacement Amplification (SDA) and Nucleic Acid Sequence-Based Amplification

(NASBA),

Rolling Circle Amplification (RCA), as well as for direct detection of targets in soln. phase or solid phase (e.g. array) assays.

L10 ANSWER 3 OF 39 CAPLUS COPYRIGHT 2001 ACS

2000:646182 Document No. 133:233560 A **method** for direct nucleic acid sequencing of single molecules using immobilized polymerases and bases labeled with a reporter dye via a photolabile linker. Stemple, Derek Lyle; Armes, Niall Antony (ASM Scientific, Inc., USA). PCT Int. Appl. WO 2000053805 A1 20000914, 50 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB873 20000310. PRIORITY: US 1999-266187 19990310.

AB The present invention provides a novel sequencing app. and the **methods** employed to det. the nucleotide sequence of many single nucleic acid mols. simultaneously, in parallel. The **methods** and app. of the present invention offer a rapid, cost effective, high through-put **method** by which nucleic acid mols. from any source can be readily sequenced without the need for prior amplification of the sample or prior knowledge of any sequence information. The **method** uses primer extension with an immobilized DNA polymerase. The **method** uses chain terminators that have the reporter moiety as a blocking group for primer extension. The polymerase is exposed in turn

to

each of the labeled bases and incorporation is detd. fluorometrically. When a dye is incorporated, it is recorded and the blocking group is then removed by photolysis. The cycle is then repeated.

L10 ANSWER 4 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

2000:305549 Document No.: PREV200000305549. Dynamics of NF kappaB and IkappaBalpha studied with green fluorescent protein (GFP) fusion proteins:

Investigation of GFP-p65 binding to DNA by fluorescence resonance energy transfer. Schmid, Johannes A.; Birbach, Andreas; Hofer-Warbinek, Renate; Pengg, Margarete; Burner, Ursul; Furtmueller, Paul G.; Binder, Bernd R.; de Martin, Rainer. Journal of Biological Chemistry, (June 2, 2000) Vol. 275, No. 22, pp. 17035-17042. print. ISSN: 0021-9258. Language: English. Summary Language: English.

AB We investigated the dynamics of nuclear transcription factor kappaB (NF-kappaB) by using fusion proteins of the p65 subunit with mutants of

green fluorescent protein (GFP). GFP-NF-kappaB chimeras were functional both in vitro and in vivo, as demonstrated by electrophoretic mobility shift assays and reporter gene studies. GFP-p65 was regulated by IkappaBalpha similar to wild type p65 and associated with its inhibitor even if both proteins were linked to a GFP protein. This finding was also verified by fluorescence resonance energy transfer (**FRET**) microscopy and studies showing mutual regulation of the intracellular localization of both GFP chimeras. Incubation of GFP-p65 with fluorescently labeled NF-kappaB-binding oligonucleotides also resulted in **FRET**. This effect was DNA sequence-specific and exhibited saturation characteristics. Application of stopped-flow fluorometry to measure the kinetics of **FRET** between GFP-p65 and oligonucleotides revealed a fast increase of acceptor fluorescence with a plateau after about 10 ms. The observed initial binding rate showed a temperature-dependent linear correlation with the oligonucleotide concentration. The association constant calculated according to pre-steady

state kinetics was $3 \times 10^6 \text{ M}^{-1}$, although equilibrium binding studies implied significantly higher values. This observation suggests that the binding process involves a rapid association with a rather high off-rate followed by a conformational change resulting in an increase of the association constant.

L10 ANSWER 5 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

2000:439111 Document No.: PREV200000439111. A fluorescence-quenched chitopentaose for the study of endo-chitinases and chitobiosidases. Cottaz, Sylvain (1); Brasme, Bernard; Driguez, Hugues. (1) CERMV-CNRS, 38041, Grenoble Cedex 9 France. European Journal of Biochemistry, (September, 2000) Vol. 267, No. 17, pp. 5593-5600. print. ISSN: 0014-2956.

Language: English. Summary Language: English.

AB A new fluorogenic substrate displaying intramolecular **fluorescence energy transfer (FRET)** has been synthesized from NI, NII, NIII, NIV-tetra-acetyl-chitopentaose. Two molecules, a fluorophore (5-(2-aminoethyl) amino-1-naphthalene-sulfonic acid; EDANS) and

a quenching group (dimethylaminophenylazophenyl; DAB) were chemically introduced on to the chitopentaose, one at each end. Among eight enzymes tested, only endo-chitinase and chitobiosidase activities could be specifically assayed by monitoring the variation of fluorescence after enzymatic hydrolysis of this substrate. Chitobioses and N-acetyl-beta-glucosaminidases are not active on the compound, the presence of a bulky chromogenic group at the 2 position of the nonreducing

end of the substrate preventing the binding and thus hydrolysis by these two exo-enzymes. The observation that chitobiosidases are able to hydrolyse a chitooligosaccharide functionalized on both extremities demonstrates the possibility of an endo-action for this class of chitinases, which are generally classified as exo-enzymes. This fluorogenic chitooligosaccharide should prove to be very useful for the detection and the convenient assay of chitinolytic activities at nanomolar concentrations.

L10 ANSWER 6 OF 39 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1

2000144736 EMBASE Coumarin-fluorescein pair as a new donor-acceptor set for **fluorescence energy transfer** study of DNA.

Mitsui T.; Nakano H.; Yamana K.. K. Yamana, Department of Applied

Chemistry, Himeji Institute of Technology, 2167 Shosha, Himeji, Hyogo 671-2201, Japan. yamana@chem.eng.himeji-tech.ac.jp. Tetrahedron Letters 41/15 (2605-2608) 8 Apr 2000.

Refs: 17.

ISSN: 0040-4039. CODEN: TELEAY.

Publisher Ident.: S 0040-4039(00)00215-X. Pub. Country: United Kingdom. Language: English. Summary Language: English.

- AB A **method** for introduction of the 2'-coumarin labeled nucleoside as a fluorescence energy donor into DNA duplexes has been described. Efficient **FRET** occurs between the coumarin-fluorescein pair in DNA owing to the high quantum yield of the donor. The present donor-acceptor pair may be useful as **FRET** indicator of DNA structures in solution. (C) 2000 Elsevier Science Ltd.

L10 ANSWER 7 OF 39 MEDLINE

DUPLICATE 2

2000304907 Document Number: 20304907. PubMed ID: 10843864.

Fluorescence energy transfer indicates similar transient and equilibrium intermediates in staphylococcal nuclease folding. Nishimura C; Riley R; Eastman P; Fink A L. (Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA, 95064, USA.) JOURNAL OF MOLECULAR BIOLOGY, (2000 Jun 16) 299 (4) 1133-46.

Journal code: J6V; 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

- AB Fluorescence resonance energy transfer (**FRET**) is one of the few **methods** available to measure the rate at which a folding protein collapses. Using staphylococcal nuclease in which a cysteine residue was engineered in place of Lys64, permitted **FRET** measurements of the distance between the donor tryptophan 140 and 5-[[2-[(iodoacetyl)-amino]ethyl]amino]naphthalene-1-sulfonic acid-labeled Cys64. These measurements were undertaken on both equilibrium partially folded intermediates at low pH (A states), as well as transient intermediates during stopped-flow refolding. The results indicate that there is an initial collapse of the protein in the deadtime of the stopped-flow instrument, corresponding to a regain of approximately 60% of the native signal, followed by three slower transients. This is in contrast to circular dichroism measurements which show only 20-25% regain of the native secondary structure in the burst phase. Thus hydrophobic collapse precedes the formation of substantial secondary structure. The first two detected transient intermediate species have **FRET** properties essentially identical with those of the previously characterized equilibrium A state intermediates, suggesting similar structures between the equilibrium and transient intermediates. The effects of anions on the folding of acid-unfolded staphylococcal nuclease, and urea on the unfolding of the resulting A states, indicates that in folding the protein becomes compact prior to formation of major secondary structure, whereas in unfolding the protein expands prior to major loss of secondary structure. Comparison of the kinetics of refolding of staphylococcal nuclease, monitored by **FRET**, and for a proline-free variant, indicate that folding occurs via two partially folded intermediates leading to a native-like species with one (or more) proline residues in a non-native conformation. For the A states an excellent correlation between compactness measured by **FRET**, and compactness determined from small-angle X-ray scattering, was observed. Further, a linear relationship between compactness and free energy of unfolding was noted. Formation of

soluble aggregates of the A states led to dramatic enhancement of the **FRET**, consistent with intermolecular **fluorescence energy transfer**.
Copyright 2000 Academic Press.

L10 ANSWER 8 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

2001:23837 Document No.: 'PREV200100023837. **Fluorescence**

energy transfer analysis of DNA structures containing several bulges and their interaction with CAP. Stuhmeier, Frank;

Hillisch,

Alexander; Clegg, Robert M. (1); Diekmann, Stephan. (1) Department of Physics and Laboratory of Fluorescence Dynamics, Loomis Laboratory of Physics, 1110 West Green Street, Urbana, IL, 61801-3080: rclegg@uiuc.edu USA. Journal of Molecular Biology, (6 October, 2000) Vol. 302, No. 5, pp. 1081-1100. print. ISSN: 0022-2836. Language: English. Summary Language: English.

AB DNA molecules with three bulges separated by double-stranded helical sections of B-DNA were constructed to be used as substrates for DNA-protein binding assays. Fluorescence resonance energy transfer (**FRET**) between dye molecules attached to the 5'-ends of the DNA molecules is used to monitor the protein binding. The A5 bulge, which consists of five unpaired adenine nucleotides, alters the direction of the helical axis by approximately 80 to 90degree at every bulge site.

Computer

molecular modeling facilitated a pre-selection of suitable helix lengths that bring the labeled ends of the three-bulge DNA molecules (60 to 70 base-pairs long) into close proximity. The **FRET** experiments verified that the labeled ends of the helices of these long molecules

were

indeed close. A series of **FRET** experiments was carried out with two A5 and two A7 bulge molecules. The relative positions of the bulges were varied along the central helical DNA sequence (between the bulges)

in

order to determine the relative angular juxtapositions of the outlying helical arms flanking the central helical region. The global structural features of the DNA molecules are manifested in the **FRET** data. The **FRET** experiments, especially those of the two-bulge series, could be interpreted remarkably well with molecular models based on the NMR structure of the A5 bulge. These models assume that the DNA molecules do not undergo large torsional conformational fluctuations at the bulge sites. The magnitude of the **FRET** efficiency attests to a relatively rigid structure for many of the long 5'-end-labeled molecules. The changes in the **FRET** efficiency of three-bulge structures containing the specific binding sequence of the catabolite activator protein (CAP) demonstrated significant deformation of the DNA upon binding

of CAP. No direct interaction of CAP with the dyes was observed.

L10 ANSWER 9 OF 39 CAPLUS COPYRIGHT 2001 ACS

2000:433850 Document No. 133:237512 fluorescence resonance energy transfer using spiropyran and diarylethene photochromic acceptors. Giordano, L.; Macareno, J.; Song, L.; Jovin, T. M.; Irie, M.; Jares-Erijman, E. A. (Departamento de Quimica Organica PROPLAME-CONICET, FCEyN, UBA, Buenos Aires, Argent.). Molecules, 5(3), 591-592 (English) 2000. CODEN:

MOLEFW.

ISSN: 1420-3049. URL: <http://www.mdpi.org/molecules/papers/50300252.pdf>
Publisher: Molecular Diversity Preservation International.

AB The prepn. and photophys. characterization were described of 2 model compds. designed to test a new approach for the quant. detn. of fluorescence resonance energy transfer (**FRET**) in biol. systems. The **method** enables modulation of **FRET** by exploiting the unique reversible spectral properties of photochromic diarylethenes and spiropyrans to create switchable energy acceptors.

L10 ANSWER 10 OF 39 MEDLINE DUPLICATE 3
2000252674 Document Number: 20252674. PubMed ID: 10790310. Red
laser-induced **fluorescence energy transfer**
in an immunosystem. Oswald B; Lehmann F; Simon L; Terpetschnig E;
Wolfbeis
O S. (Institute of Analytical Chemistry, Chemo- and Biosensors,
University
of Regensburg, Regensburg, 93040, Germany.) ANALYTICAL BIOCHEMISTRY,
(2000 May 1) 280 (2) 272-7. Journal code: 4NK; 0370535. ISSN: 0003-2697.
Pub. country: United States. Language: English.

AB We describe two near-infrared fluorescent squaraine dyes (Sq635 and Sq660), their spectra, their covalent linkage to proteins, and their use as donor and acceptor, respectively, in a fluorescence resonance energy transfer (**FRET**) immunoassay based on the use of red lasers. The dyes show quantum yields of around 10% in the free form and up to 68% when bound to proteins. If converted into their N-hydroxysuccinimide esters, they can be linked to free amino groups of proteins. To improve water solubility, two sulfo groups were introduced. The emission spectrum of Sq635 overlaps the absorption spectrum of Sq660, a fact that makes them a useful pair of dyes for use in **FRET** immunoassay which is demonstrated for human serum albumin/anti-human serum albumin. Copyright 2000 Academic Press.

L10 ANSWER 11 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS
2000:184868 Document No.: PREV200000184868. PKC membrane association and dissociation kinetics studied by **fret** in living PC12 cell.
Janoshazi, Agnes (1); Geeraert, Virginie; Dupont, Jean-Luc; de Barry, Jean. (1) CNRS, Strasbourg, Cell Neurobiology Laboratory, University
Louis
Pasteur, 4, Blaise Pascal, BAS-RHIN, Strasbourg, 67000 France.
Biophysical
Journal, (Jan., 2000) Vol. 78, No. 1 Part 2, pp. 248A. Meeting Info.:
44th
Annual Meeting of the Biophysical Society. New Orleans, Louisiana, USA
February 12-16, 2000 ISSN: 0006-3495. Language: English. Summary
Language:
English.

L10 ANSWER 12 OF 39 MEDLINE DUPLICATE 4
2001178131 Document Number: 21063624. PubMed ID: 11121303. ATP-induced transconformation of myosin revealed by determining three-dimensional positions of fluorophores from **fluorescence energy transfer** measurements. Yasunaga T; Suzuki Y; Ohkura R; Sutoh K; Wakabayashi T. (Department of Physics, School of Science, University of Tokyo, 7-3-1, Hongo, Bunkyo, Tokyo 113-0033, Japan.) JOURNAL OF STRUCTURAL BIOLOGY, (2000 Oct) 132 (1) 6-18. Journal code: AUD; 9011206. ISSN: 1047-8477. Pub. country: United States. Language: English.
AB The **method** of fluorescence resonance energy transfer (**FRET**) is one of the most important techniques for measuring the distance between two fluorophores and for detecting the changes in protein

structure under physiological conditions. The use of green fluorescent protein is also a powerful technology that has been used to elucidate dynamic molecular events. From these we have developed a novel **method** to determine the three-dimensional positions of fluorophores by combining the **FRET** data and other structural information available. Using this **method**, we could determine the ATP-induced changes of three-dimensional structure of truncated Dictyostelium myosin in solution. The myosin structure with ADP in solution was found to be similar to that of the crystal structure of MgADPBeFx-bound truncated Dictyostelium myosin (type I structure), whereas myosin with ATP in solution was similar to the crystal structure of MgAdPVi-bound one (type II structure). However, the crystal structure of MgADP-bound scallop myosin (type III structure) could not be explained by any of our **FRET** data under various conditions. This indicates that the type III crystal structure might represent a transient intermediate conformation that could not be detected using **fluorescence energy transfer**. Copyright 2000 Academic Press.

L10 ANSWER 13 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

2000:429568 Document No.: PREV200000429568. Intradomain distances in the regulatory domain of the myosin head in prepower and postpower stroke states: **Fluorescence energy transfer**. Palm, Thomas; Sale, Ken; Brown, Louise; Li, Huichun; Hambly, Brett; Fajer, Peter

G. (1). (1) National High Magnetic Field Laboratory, Department of Biological Science, Institute of Molecular Biophysics and Florida State University, Tallahassee, FL, 32306 USA. Biochemistry, (Oct. 5, 1999) Vol. 38, No. 40, pp. 13026-13034. print. ISSN: 0006-2960. Language: English. Summary Language: English.

AB The relative movement of the catalytic and regulatory domains of the myosin head (S1) is likely to be the force generating conformational change in the energy transduction of muscle (Rayment, I., Holden, H. M., Whittaker, M., Yohn, C. B., Lorenz, M., Holmes, K. C., and Milligan, R.

A. (1993) Science 261, 58-65). To test this model we have measured, using frequency-modulated **FRET**, three distances between the catalytic domain and regulatory domains and within the regulatory domain of myosin. The donor/acceptor pairs included MHC cys707 and ELC cys177; ELC cys177 and RLC cys154; and ELC cys177 and gizzard RLC cys108. The IAEDANS (donor)

or acceptor (DABMI or IAF) labeled light chains (ELC and RLC) were exchanged into monomeric myosin and the distances were measured in the putative prepower stroke states (in the presence of MgATP or ADP/AlF4-) and the postpower stroke states (ADP and the absence of nucleotides). For each of the three distances, the donor/acceptor pairs were reversed to minimize uncertainty in the distance measured, arising from probe orientational factors. The distances obtained from **FRET** were in close agreement with the distances in the crystal structure. Importantly, none of the measured distances varied by more than 2 ANG, putting a

strong constraint on the extent of conformational changes within S1. The maximum axial movement of the distal part of myosin head was modeled using **FRET** distance changes within the myosin head reported here and previously. These models revealed an upper bound of 85 ANG for a swing of the regulatory domain with respect to the catalytic domain during the power stroke. Additionally, an upper bound of 22 ANG could be contributed

to the power stroke by a reorientation of RLC with respect to the ELC during the power stroke.

L10 ANSWER 14 OF 39 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 5
1999242024 EMBASE Synthesis and properties of oligonucleotide duplexes containing donor and acceptor fluorophores at 2'-positions. Yamana K.; Mitsui T.; Nakano H.. K. Yamana, Department of Applied Chemistry, Himeji Institute of Technology, 2167 Shosha, Himeji, Hyogo 671-2201, Japan. yamana@chem.eng.himeji-tech.ac.jp. Tetrahedron 55/30 (9143-9150) 23 Jul 1999.

Refs: 32.

ISSN: 0040-4020. CODEN: TETRAB.

Publisher Ident.: S 0040-4020(99)00484-6. Pub. Country: United Kingdom.

Language: English. Summary Language: English.

AB A **method** for introduction of dimethylamionaphthamide and fluorescein labels as a fluorescence energy donor and acceptor pair into 2'-positions of DNA duplexes has been described. It has been shown that the attachment of these bulky fluorophores to the sugar 2'-position at the

terminal fraying end of each oligonucleotide strand does not alter the normal thermal stability and global conformation of the DNA duplexes. A clear dependence of **fluorescence energy transfer** efficiency on the number of nucleotides in DNA has been observed, suggesting that the present donor-acceptor pair may be useful for **FRET** indicator of DNA.

L10 ANSWER 15 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

1999:134135 Document No.: PREV199900134135. **Fluorescence**

energy transfer as a probe for tetraplex formation: The i-motif. Mergny, Jean-Louis (1). (1) Laboratoire de Biphysique, Museum National d'Histoire Naturelle, INSERM U 201, CNRS URA 481, 43 rue Cuvier, 75005 Paris France. Biochemistry, (Feb. 2, 1999) Vol. 38, No. 5, pp. 1573-1581. ISSN: 0006-2960. Language: English.

AB The secondary structure of cytosine-rich oligodeoxynucleotides has been investigated with fluorescent probes. Intramolecular folding of an oligonucleotide into an i-DNA motif led to fluorescence excitation energy transfer between a donor (fluorescein) and an acceptor (tetramethylrhodamine) covalently attached to the 5' and 3' ends of the DNA, respectively, provided that a suitable linker was chosen. The conjugation of the dyes to the oligonucleotide had an influence on the thermodynamics of i-motif formation as well as on the kinetics of folding. Intramolecular folding was demonstrated from the concentration independence of **FRET** over a wide concentration range. Folding of the oligonucleotide was confirmed by UV absorption melting experiments. The folding of the i-motif could be followed at concentrations as low as 50 pM. **Fluorescence energy transfer** can thus be used to reveal the formation of multistranded DNA structures.

L10 ANSWER 16 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

1999:483223 Document No.: PREV199900483223. Oligonucleotides labeled by fluorophores at the sugar 2'-positions for **fluorescence energy transfer** study of nucleic acid structure. Yamana, Kazushige (1); Mitsui, Tsuneo. (1) Department of Applied Chemistry, Himeji

Institute of Technology, 2167 Shosha, Himeji, 671-2201 Japan. Nucleosides & Nucleotides, (June July, 1999) Vol. 18, No. 6-7, pp. 1565-1566. ISSN: 0732-8311. Language: English. Summary Language: English.

AB Oligonucleotides containing 2'-(6-dimethylamino-2-naphthamide)uridine have

been shown to be useful as a donor fluorophore in **FRET** to oligonucleotides labeled with fluorescein at the 2'-position as an acceptor molecule.

L10 ANSWER 17 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

1999:310703 Document No.: PREV199900310703. **FRET**-based intra-tRNA distance measurements for nucleocapsid protein-dependent tRNA unwinding during priming of HIV reverse transcription. Musier-Forsyth, K. (1);

Chan,

B. (1); Weidemaier, K. (1); Yip, W.-T. (1); Barbara, P. F. (1). (1) University of Minnesota, Minneapolis, MN USA. FASEB Journal, (April 23, 1999) Vol. 13, No. 7, pp. A1319. Meeting Info.: Annual Meeting of the American Societies for Experimental Biology on Biochemistry and Molecular Biology 99 San Francisco, California, USA May 16-20, 1999 American Societies for Experimental Biology. ISSN: 0892-6638. Language: English.

L10 ANSWER 18 OF 39 CAPLUS COPYRIGHT 2001 ACS

1999:673404 Document No. 131:348227 Fluorescence lifetime imaging of receptor tyrosine kinase activity in cells. Wouters, Fred S.; Bastiaens, Philippe I. H. (Cell Biophysics Laboratory, Imperial Cancer Research

Fund,

London, WC2A 3PX, UK). Curr. Biol., 9(19), 1127-1130 (English) 1999. CODEN: CUBLE2. ISSN: 0960-9822. Publisher: Current Biology

Publications.

AB We report a highly specific fluorescence lifetime imaging microscopy (FLIM) **method** for monitoring epidermal growth factor receptor (EGFR) phosphorylation in cells based on fluorescence resonance energy transfer (**FRET**). EGFR phosphorylation was monitored using a green fluorescent protein (GFP)-tagged EGFR and Cy3-conjugated anti-phosphotyrosine antibodies. In this **FRET**-based imaging **method**, the information about phosphorylation is contained only in the (donor) GFP fluorescence lifetime and is independent of the antibody-derived (acceptor) fluorescence signal. A pixel-by-pixel ref. lifetime of the donor GFP in the absence of **FRET** was acquired from the same cell after photobleaching of the acceptor. We show that this calibration, by acceptor photobleaching, works for the GFP-Cy3 donor-acceptor pair and allows the full quantitation of **FRET** efficiencies, and therefore the degree of exposed phosphotyrosines, at each pixel. The hallmark of EGFR stimulation is receptor dimerization

and

concomitant activation of its intracellular tyrosine kinase domain. Trans-autophosphorylation of the receptor on specific tyrosine residues couples the activated dimer to the intracellular signal transduction machinery as these phosphorylated residues serve as docking sites for adaptor and effector mols. contg. Src homol. 2 (SH2) and phosphotyrosine-binding (PTB) domains. The time-course and extent of

EGFR

phosphorylation are therefore important determinants of the underlying pathway and resulting cellular response. Our results strongly suggest that secondary proteins are recruited by activated receptors in

endosomes,

indicating that these are active compartments in signal transduction.

L10 ANSWER 19 OF 39 MEDLINE

2000241381 Document Number: 20241381. PubMed ID: 10780469. Efficiency enhancement of long-range energy transfer by sequential multistep **FRET** using fluorescence labeled DNA. Kawahara S; Uchimaru T; Murata S. (Department of Physical Chemistry, National Institute of

Materials and Chemical Research, Agency of Industrial Science and Technology, MITI, Tsukuba Science City, Japan.) NUCLEIC ACIDS SYMPOSIUM SERIES, (1999) (42) 241-2. Journal code: O8N; 8007206. ISSN: 0261-3166. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The efficiency of long-range (ca. 80 Å) **fluorescence energy transfer** was enhanced about 1.5 times by a third chromophore located midway between two chromophores. A third chromophore should act like a relay station in sequential multistep energy transfer.

L10 ANSWER 20 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

1999:193563 Document No.: PREV199900193563. Membrane organization of a GPI-anchored protein during transcytosis revealed by imaging **fluorescence energy transfer (FRET)**

measurements. Kenworthy, A. K. (1); Hubbard, A. L.; Edidin, M. (1). (1) Dept. of Biology, Johns Hopkins University, Baltimore, MD, 21218 USA. Biophysical Journal, (Jan., 1999) Vol. 76, No. 1 PART 2, pp. A232.

Meeting

Info.: Forty-third Annual Meeting of the Biophysical Society Baltimore, Maryland, USA February 13-17, 1999 ISSN: 0006-3495. Language: English.

L10 ANSWER 21 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

2000:41935 Document No.: PREV200000041935. The use of EGFP fusion constructs to analyze cytokine factor receptor activation in vivo by fluorescence microscopy and **FRET (fluorescence energy transfer)** based applications. Jahn, Thomas (1); Coutinho, Sunita

(1); Seipel, Petra (1); Peschel, Christian (1); Duyster, Justus (1). (1) Department of Internal Medicine III, Technical University of Munich, Munich Germany. Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 46a. Meeting Info.: Forty-first Annual Meeting of the American Society

Society

of Hematology New Orleans, Louisiana, USA December 3-7, 1999 The American Society of Hematology. ISSN: 0006-4971. Language: English.

L10 ANSWER 22 OF 39 CAPLUS COPYRIGHT 2001 ACS

1998:293654 Document No. 128:317992 **Method** for detecting point mutations in dna utilizing **fluorescence energy transfer**.

Parkhurst, Lawrence J.; Parkhurst, Kay M.; Middendorf, Lyle (University of Nebraska at Lincoln, USA). PCT Int. Appl. WO 9818965 A1 19980507, 27 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US19525 19971028. PRIORITY: US 1996-29775 19961029.

AB A **method** for detecting point mutations in DNA using a fluorescently labeled oligomeric probe and fluorescence resonance energy transfer (**FRET**) is disclosed. The selected probe is initially labeled at each end with a fluorescence dye, which act together as a donor/acceptor pair for **FRET**. The fluorescence emission from the dyes changes dramatically from the duplex stage, wherein the probe is hybridized to the complementary strand of DNA, to the single-strand stage,

when the probe is melted to become detached from the DNA. The change in fluorescence is caused by the dyes coming into closer proximity after melting occurs and the probe becomes detached from the DNA strand. The change in fluorescence emission as a function of temp. is used to calc.

base the melting temp. of the complex or Tm. In the case where there is a mismatch between the probe and the DNA strand, indicating a point mutation, the Tm has been found to be significantly lower than the Tm for a perfectly match probe/target duplex. The present invention allows for the detection of the existence and magnitude of Tm, which allows for the quick and accurate detection of a point mutation in the DNA strand and, in some applications, the detn. of the approx. location of the mutation within the sequence. Oligodeoxyribonucleotide probes (10-30 nucleotides) and rhodamine or fluorescein dyes can be used in the process.

L10 ANSWER 23 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

1998:296882 Document No.: PREV199800296882. Identification of a model cardiac glycoside receptor: Comparisons with Na⁺,K⁺-ATPase. Kasturi, Rama; Yuan, Jie; McLean, Larry R.; Margolies, Michael N.; Ball, William J., Jr. (1). (1) Dep. Pharmacol. and Cell Biophys., Univ. Cincinnati Coll. Med., 231 Bethesda Ave., Cincinnati, OH 45267-0524 USA. Biochemistry, (May 12, 1998)

Vol. 37, No. 19, pp. 6658-6666. ISSN: 0006-2960. Language: English.

AB The availability of high-affinity anti-digoxin monoclonal antibodies (mAbs) offers the potential for their use as models for the characterization of the relationship between receptor structure and cardiac glycoside binding. We have characterized the binding of anthroylouabain (AO), a fluorescent derivative of the cardiac glycoside ouabain, to mAbs 26-10, 45-20, and 40-50 (Mudgett-Hunter, M., et al. (1995) Mol. Immunol. 22, 477) and lamb kidney Na⁺,K⁺-ATPase by monitoring the resultant AO fluorescence emission spectra, anisotropy, lifetime values, and Forster resonance energy transfer (**FRET**) from protein tryptophan(s) (Trp) to AO. These data suggest that the structural environment in the vicinity of the AO binding site of Na⁺,K⁺-ATPase is similar to that of mAb 26-10 but not mAbs 45-20 and 40-50. A model of AO complexed to the antigen binding fragment (Fab) of mAb 26-10 which was generated using known X-ray crystal structural data (Jeffrey, P. D., et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 10310) shows a heavy chain Trp residue (Trp-H100) that is close (apprx3 ANG) to the anthroyl moiety. This is consistent with the energy transfer seen upon AO binding to mAb 26-10 and suggests that Trp-H100, which is part of the antibody's cardiac glycoside binding site, is a major determinant of the fluorescence properties of bound AO. In contrast, the generated model of AO complexed to Fab 40-50 (Jeffrey, P. D., et al. (1995) J. Mol. Biol. 248, 344) shows a heavy chain Tyr residue (Tyr-H100) which is part of the cardiac glycoside binding site, located apprxl0 ANG from the anthroyl moiety. The closest Trp residues (H52 and L35) are located apprxl7 ANG from the anthroyl moiety, and no **FRET** is observed despite the fact that these Trp residues are close enough for significant **FRET** to occur. The energy transfer seen upon AO binding to Na⁺,K⁺-ATPase suggests the presence of one completely quenched or two highly quenched enzyme Trp residues apprxl0 and apprxl7 ANG, respectively, from the anthroyl moiety. These data suggest that the Na⁺,K⁺-ATPase Trp residue(s) involved in **fluorescence energy transfer** to AO is likely to be part of the cardiac glycoside binding site.

L10 ANSWER 24 OF 39 MEDLINE

DUPLICATE 6

1998186848 Document Number: 98186848. PubMed ID: 9518501. Detection of programmed cell death using **fluorescence energy transfer**. Xu X; Gerard A L; Huang B C; Anderson D C; Payan D G; Luo Y. (Rigel, Inc., 772 Lucerne Drive, Sunnyvale, CA 94086, USA.)

NUCLEIC ACIDS RESEARCH, (1998 Apr 15) 26 (8) 2034-5. Journal code: O8L; 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom.

Language:

English.

AB **Fluorescence energy transfer (FRET)**

) can be generated when green fluorescent protein (GFP) and blue fluorescent protein (BFP) are covalently linked together by a short peptide. Cleavage of this linkage by protease completely eliminates **FRET** effect. Caspase-3 (CPP32) is an important cellular protease activated during programmed cell death. An 18 amino acid peptide containing CPP32 recognition sequence, DEVD, was used to link GFP and BFP together. CPP32 activation can be monitored by **FRET** assay during the apoptosis process.

L10 ANSWER 25 OF 39 SCISEARCH COPYRIGHT 2001 ISI (R)

1998:581115 The Genuine Article (R) Number: 103XD. Mapping the binding sites of peptide and non-peptide molecules to G protein-coupled receptors by fluorescence. Chollet A (Reprint); Turcatti G. GLAXO WELLCOME INC,

GENEVA

BIOMED RES INST, CH-1228 GENEVA, SWITZERLAND. LETTERS IN PEPTIDE SCIENCE (MAY 1998) Vol. 5, No. 2-3, pp. 79-82. Publisher: KLUWER ACADEMIC PUBL. SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS. ISSN: 0929-5666. Pub. country: SWITZERLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Novel fluorescence approaches to investigate ligand recognition and structure of G protein-coupled receptors in native membranes have been developed. These **methods** combine the biosynthetic incorporation of unnatural fluorescent amino acids at known sites in receptors with the technique of **fluorescence energy transfer** for distance measurement. This permits one to fix the ligand in space and to define the structure of the receptor in a model of ligand-receptor interactions. Subdomains of ligand binding sites on NK1 and NK2 receptors were also characterized using environment-sensitive fluorophores and the techniques of collisional quenching and anisotropy. Antagonists and agonists have different binding sites on NK1 and NK2.

L10 ANSWER 26 OF 39 MEDLINE

97263767 Document Number: 97263767. PubMed ID: 9109682. Fluorescence resonance energy transfer study of shape changes in membrane-bound bovine prothrombin and meizothrombin. Chen Q; Lentz B R. (Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill 27599, USA.) BIOCHEMISTRY, (1997 Apr 15) 36 (15) 4701-11. Journal code: AOG; 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Prothrombin activation to thrombin is a key control reaction in blood coagulation. During the process, prothrombin is sequentially cleaved at two peptide bonds (Arg323-Ile and Arg274-Thr) by factor X(a) to generate meizothrombin and then thrombin. Phosphatidylserine (PS)-containing membranes from platelets are believed to facilitate this two-step process.

Using **fluorescence energy transfer (FRET)**, we determined the distances of closest approach between a specifically located C-terminal fluorescein of a double mutant bovine prothrombin (P(S528A, G581C)-FM) or meizothrombin (M(S528A, G581C)-FM)

and

phosphatidylethanolamine-N-rhodamine B (PE-Rh; 0-8.7 mol %) contained in membranes composed of PS (25 mol %) and phosphatidylcholine (66.3-75 mol %). Plots of the energy transfer efficiency as a function of membrane

concentration, at six PE-Rh surface densities, were analyzed globally to obtain dissociation constants and binding stoichiometries as global parameters and saturating energy transfer efficiencies characteristic of each surface density. From the global analysis, the dissociation constants were estimated to be 0.32 ± 0.10 and 0.28 ± 0.12 μM with stoichiometries of 42 ± 12 and 44 ± 9 lipid/protein for prothrombin and meizothrombin, respectively. The distance of closest approach was obtained from the dependence of the saturating energy transfer efficiency on the acceptor (PE-Rh) surface density. With the assumptions of $\kappa^2 = 2/3$ and $n = 1.4$, the distances were 94 ± 3 Å for prothrombin and 114 ± 2 Å for meizothrombin. Since both prothrombin and meizothrombin behave in solution as oblate ellipsoids of revolution with a long axis of 120 Å, our FRET measurements suggest that binding to PS-containing membranes induced tighter folding of the prothrombin molecule but not of the meizothrombin intermediate. This observation is consistent with our hypothesis that membrane binding plays an essential role in the sequential alignment of the bond Arg323-Ile in prothrombin and Arg274-Thr in meizothrombin with the active site of the membrane-bound prothrombinase in the two-step thrombin-generating process.

L10 ANSWER 27 OF 39 MEDLINE DUPLICATE 7
 97395702 Document Number: 97395702. PubMed ID: 9251806. Interhead distances in myosin attached to F-actin estimated by **fluorescence energy transfer** spectroscopy. Ishiwata S; Miki M; Shin I; Funatsu T; Yasuda K; dos Remedios C G. (Department of Physics, School of Science and Engineering, Waseda-University, Japan.. ishiwata@mn.waseda.ac.jp) . BIOPHYSICAL JOURNAL, (1997 Aug) 73 (2) 895-904. Journal code: A5S; 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB Fluorescence resonance energy transfer (**FRET**) spectroscopy has been used to determine distances between probes attached to the most reactive sulfhydryl (SH1) group on individual myosin "heads." We measured intramolecular and intermolecular interhead distances as well as the distance between one head of heavy meromyosin (HMM) mixed with subfragment-1 (S1) heads attached to F-actin under rigor conditions. The SH1 cysteine was specifically labeled with either a donor (5-(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid) or an acceptor probe (5-iodoacetamidofluorescein). In free solution, the distance between these probes was too large to allow significant **FRET**, but in the rigor complex with F-actin, intermolecular interhead distances between S1 molecules, HMM molecules, or S1 and HMM were determined to be 6.0-6.3 nm. The radial coordinate of the labels relative to F-actin was 5.0-6.4 nm. However, the intramolecular interhead distance in HMMs in which the two heads were labeled with D and A probes was estimated to be larger. The binding affinity of the second head of HMM(D/A) to F-actin may be reduced because of heterogeneous modification of the SH1 groups, such that the probability of single-head binding is increased.

L10 ANSWER 28 OF 39 MEDLINE DUPLICATE 8
 1998039886 Document Number: 98039886. PubMed ID: 9372617.
Fluorescence energy transfer-sensitized photobleaching of a fluorescent label as a tool to study donor-acceptor

distance distributions and dynamics in protein assemblies: studies of a complex of biotinylated IgM with streptavidin and aggregates of concanavalin A. Mekler V M; Averbakh A Z; Sudarikov A B; Kharitonova O V. (Institute of Chemical Physics, Russian Academy of Sciences, Chernogolovka, Moscow Region, Russia.. mekler@icp.ac.ru) . JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY. B, BIOLOGY, (1997 Oct) 40 (3) 278-87. Journal code: JLI; 8804966. ISSN: 1011-1344. Pub. country: Switzerland. Language: English.

AB A photokinetic **method** of detection of fluorescence resonance energy transfer (**FRET**) between special fluorescent labels is applied to study time-averaged spatial distribution of labeled proteins

in protein assemblies. Prolonged irradiation of a sample at the absorption maximum of the energy donor label initiates **FRET**-sensitized fluorescence photobleaching of the energy acceptor label, which was monitored by steady-state fluorimetric measurements. Kinetics of the acceptor photobleaching and kinetics of decreasing the efficiency of **FRET** from donors to unbleached acceptors were determined. The **FRET** efficiency was found from measuring sensitization of acceptor fluorescence. Analysis of the photokinetic data permits to estimate the time-averaged distribution of acceptors on donor-acceptor distances in

the range of characteristic distances of **FRET**. Dynamic processes influencing donor-acceptor distances can be also investigated by the **method**. Application of the **method** is demonstrated by the studies of a complex of biotinylated IgM with streptavidin and aggregates composed of concanavalin A and sodium dodecyl sulphate. A new thiadicarbocyanine dye was used as the acceptor label, R-phycoerythrin

and tetramethylrhodamine isothio-cyanate were the donor labels. In the IgM-streptavidin complex, 16% of acceptors most contributed to **FRET** provided 90% of **FRET** efficiency, whereas acceptors made about the same time-averaged contribution to **FRET** in the concanavalin A aggregates.

L10 ANSWER 29 OF 39 CAPLUS COPYRIGHT 2001 ACS

1997:28384 Document No. 126:99924 Detection of point mutations in DNA by **fluorescence energy transfer**. Parkhurst, Kay M.; Parkhurst, Lawrence J. (Department of Chemistry, University of Nebraska, Lincoln, NE, 68588-0304, USA). J. Biomed. Opt., 1(4), 435-441 (English) 1996. CODEN: JBOPFO. ISSN: 1083-3668. Publisher: SPIE-The International Society for Optical Engineering.

AB A **method** has been developed for the rapid and direct identification of a single point mutation in a DNA sequence using fluorescence resonance energy transfer (**FRET**). The probe was a 16-base oligomer with 5'-bound x-rhodamine and 3'-bound fluorescein (R*16*F); the two dyes acted as a donor/acceptor pair for **FRET**, resulting in a dramatic difference in the fluorescence emission of the R*16*F in a duplex structure (hybridized to a complementary strand) and

as a single strand (melted). This difference was used to obtain the melting temp. (Tm), by spectroscopically following the transition from double to single strand, for the probe hybridized to three different strands: the 16-base complement, the 16-base complement contg. a single base mismatch, and the 16-base complementary sequence in the phage DNA M13mp18(+). The Tms thus detd. for the perfectly base-paired duplexes, with R*16*F hybridized to the 16-mer complement and to M13, differed by 2.degree., whereas the Tm obtained for R*16*F hybridized to the mismatched 16-mer

complement was 10.degree. lower than that for the perfect duplex. The sharpness of the transition and the ease of detection allow single base mismatches to be reliably detected in nano- and subnanomolar concns. in less than 1 h following hybridization.

L10 ANSWER 30 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS
1997:146020 Document No.: PREV199799445223. Degradation of oligonucleotides in

the anti-gene strategy: A **FRET (fluorescence energy transfer)** study. Refregiers, M.; Jolles, B.; Chinsky, L.; Laigle, A.. LPBC, ESTER, Univ. P. and M Curie, Paris France. Progress in Biophysics and Molecular Biology, (1996) Vol. 65, No. SUPPL. 1, pp. 73. Meeting Info.: XIIth International Biophysics Congress Amsterdam, Netherlands August 11-16, 1996 ISSN: 0079-6107. Language: English.

L10 ANSWER 31 OF 39 MEDLINE DUPLICATE 9
96170587 Document Number: 96170587. PubMed ID: 8600827.

Fluorescence energy transfer immunoassay based on a long-lifetime luminescent metal-ligand complex. Youn H J; Terpetschnig E; Szmazinski H; Lakowicz J R. (Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore 21201, USA.) ANALYTICAL BIOCHEMISTRY, (1995 Nov 20) 232 (1) 24-30. Journal code: 4NK; 0370535. ISSN: 0003-2697. Pub. country: United States. Language: English.

AB We describe an immunoassay based on fluorescence resonance energy transfer

(**FRET**). The antigen was human serum albumin (HSA), which was labeled with a ruthenium-ligand complex, [Ru(bpy)₂(phen-ITC)]²⁺. The antibody (IgG) to HSA was labeled with a nonfluorescent absorber,

Reactive

Blue 4. Association of the Ru-labeled HSA with the antibody was detected by three spectral parameters, a decreased quantum yield of Ru-HSA, a decrease in its fluorescence lifetime, and an increase in its

fluorescence

anisotropy. The steady-state anisotropy of Ru-HSA increased approximately eightfold upon binding to the antibody. These spectral effects were observed both in the direct association of the Ru-HSA with Reactive Blue 4-labeled antibody, and in a competitive assay format wherein unlabeled HSA competed with Ru-HSA for the binding sites on the antibody. Some nonspecific interactions of HSA may have occurred with Reactive Blue 4-labeled AHA, a difficulty which can be avoided with a different acceptor. The use of **FRET** provides a reliable means to alter the spectral properties upon antigen-antibody binding. The advantages of a ruthenium-ligand fluorophore include its long-wavelength absorption and emission, long fluorescence lifetime, and high photo-stability. Long wavelengths minimize problems of autofluorescence from biological

samples,

and long life-times allow off-gating of the prompt autofluorescence.

L10 ANSWER 32 OF 39 CAPLUS COPYRIGHT 2001 ACS

1995:350854 Document No. 122:127536 **Fluorescence energy transfer** substrates for protease determination. Garman, Andrew John (Zeneca Ltd., UK). PCT Int. Appl. WO 9428166 A1 19941208, 22 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK,

ES,

FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN; RW: AT, BE,

BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2.
APPLICATION: WO 1994-GB1153 19940527. PRIORITY: GB 1993-10978 19930527.

AB A **method** for the prepn. of a fluorescence resonance energy transfer (**FRET**) substrate having donor and acceptor species on opposite sides of a proteolytic cleavage site and wherein the donor and/or acceptor species are attached via the side chain(s) of amino acid(s) therein is described. The **method** comprises contacting a reactive donor or acceptor species with a polypeptide substrate having the side chain(s) of amino acid(s) therein adapted for reaction with the reactive species and then contacting the substrate so obtained with a corresponding reactive donor or acceptor species. Novel **FRET** substrates so prepd. and their use in assays to identify modulators of protease activity are claimed. Substrates of endothelin converting enzyme (ECE) and of staphylococcal V8 protease were modified as described and assayed for substrate activity and usefulness. Thus, the amino terminus of human 16-37-[Cys-26]big endothelin-1 was reacted with 5-maleimidofluorescein then with tetramethylrhodamine isothiocyanate to prepd. the **FRET** substrate for ECE.

L10 ANSWER 33 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS
1995:68838 Document No.: PREV199598083138. Single-turnover kinetics of helicase-catalyzed DNA unwinding monitored continuously by **fluorescence energy transfer**. Bjornson, Keith P.; Amaratunga, Mohan; Moore, Keith J. M.; Lohman, Timothy M. (1). (1) Dep. Biochem., Mol. Biophysics, Washington University Sch. Med., Box 8231, 660 S. Euclid Ave., St. Louis, MO 63110 USA. Biochemistry, (1994) Vol. 33,

No. 47, pp. 14306-14316. ISSN: 0006-2960. Language: English.

AB We describe a fluorescence assay that can be used to monitor helicase-catalyzed unwinding of duplex DNA continuously in real time. The assay is based on the observation that fluorescence resonance energy transfer (**FRET**) occurs between donor (fluorescein) and acceptor (hexachlorofluorescein) fluorophores that are in close proximity due to their covalent attachment to the 3' and 5' ends of the complementary strands of a duplex oligodeoxynucleotide. **FRET** results in a reduction in the fluorescence emission intensity of fluorescein in the duplex DNA substrate relative to that observed for fluorescein-labeled single stranded DNA. Therefore, an enhancement of fluorescein fluorescence

($\lambda_{\text{exc}} = 492 \text{ nm}$; $\lambda_{\text{em}} = 520 \text{ nm}$) occurs upon helicase-catalyzed unwinding of the duplex DNA and separation of the complementary strands. The fluorescence assay is extremely sensitive, allowing DNA unwinding reactions to be monitored continuously at DNA concentrations as low as 1 nM in a fluorescence stopped-flow experiment. We demonstrate the use of this DNA substrate in pre-steady state, single turnover studies of duplex DNA unwinding catalyzed by the Escherichia coli Rep helicase, monitored

by fluorescence stopped flow. We show that the fluorescence enhancement monitors Rep-catalyzed DNA unwinding by comparisons with identical kinetic

studies carried out using rapid chemical quench-flow techniques. Single turnover kinetic studies performed at 1 nM DNA as a function of excess Rep

concentration show that Rep-catalyzed unwinding of an 18 base pair duplex containing a 3'-ss-(dT)-20 tail is biphasic and can be described by the sum of two exponential terms. The observed rate constant of the first phase is independent of (Rep) (20-300 nM) and measures the rapid single turnover unwinding of the duplex DNA by Rep dimers bound in productive complexes (1.3 +/- 0.2 s⁻¹; 23 +/- 3 base pairs s⁻¹ at 25.0 degree C). The observed rate constant for the second phase increases linearly with

(Rep),

reflecting DNA unwinding that is limited by a Rep binding event occurring with a bimolecular rate constant of (1.8 +/- 0.1) times 10⁵ M⁻¹ s⁻¹, which may reflect the rate constant for Rep dimerization on DNA. Kinetic competition studies indicate that both Rep subunits are bound stably to the DNA substrate in the productive complex that is unwound in the fast phase. The results of these kinetic studies are consistent with an

active,

rolling mechanism for Rep-catalyzed unwinding of DNA (Wong, I., & Lohman, T. M., (1992) Science 256, 350). This fluorescence assay should greatly facilitate further mechanistic studies of helicase-catalyzed DNA unwinding.

L10 ANSWER 34 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS

1995:16409 Document No.: PREV199598030709. Luminescence energy transfer using a terbium chelate: Improvements on **fluorescence energy transfer**. Selvin, Paul R. (1); Hearst, John E.. (1) Dep. Chem., Univ. California, Structural Biol. Div., Lawrence Berkeley Lab., Berkely, CA 94720 USA. Proceedings of the National Academy of Sciences of the United States of America, (1994) Vol. 91, No. 21, pp. 10024-10028. ISSN: 0027-8424. Language: English.

AB We extend the technique of fluorescence resonance energy transfer (**FRET**) by introducing a luminescent terbium chelate as a donor and an organic dye, tetramethylrhodamine, as an acceptor. The results are consistent with a Forster theory of energy transfer, provided the appropriate parameters are used. The use of lanthanide donors, in general,

and this pair, in particular, has many advantages over more conventional **FRET** pairs, which rely solely on organic dyes. The distance at which 50% energy transfer occurs is large, 65 Å; the donor lifetime is a single exponential and long (millisecond), making lifetime measurements facile and accurate. Uncertainty in the orientation factor, which creates uncertainty in measured distances, is minimized by the donor's multiple electronic transitions and long lifetime. The sensitized emission of the acceptor can be measured with little or no interfering background, yielding a gt 25-fold improvement in the signal-to-background ratio over standard donor-acceptor pairs. These improvements are expected to make distances gt 100 Å measurable via **FRET**. We also report measurement of the sensitized emission lifetime, a measurement that is completely insensitive to total concentration and incomplete labeling.

L10 ANSWER 35 OF 39 MEDLINE

DUPLICATE 10

95069047 Document Number: 95069047. PubMed ID: 7978374.

Fluorescence energy transfer shows that various physical and chemical treatments of human sperm induce unpacking of chromatin. Zuccotti M; Katayose H; Matsuda J; Redi C A; Bottirolì G; Yanagimachi R. (Dipartimento di Biologia Animale, Università di Pavia, Italy.) ANDROLOGIA, (1994 Jul-Aug) 26 (4) 225-30. Journal code: 4QP; 0423506. ISSN: 0303-4569. Pub. country: GERMANY: Germany, Federal

Republic

of. Language: English.

AB Fluorescence resonance energy transfer (**FRET**) was used to study the changes which human sperm chromatin went through after various physical and chemical treatments. This technique showed a dilatation of the spatial relationship among chromatin linear arrays, with UV and DNase among the treatments that gave rise to the highest increase. **FRET** image analysis showed that the chromatin linear arrays after treatment reach a spatial disarrangement similar to that brought about by sperm decondensation. Comparison of these results with the ability of human treated sperm to form pronuclei after microinjection into hamster eggs, suggests that the highly condensed spatial organization of sperm chromatin arrays may not be a necessary prerequisite for pronucleus formation.

L10 ANSWER 36 OF 39 CAPLUS COPYRIGHT 2001 ACS
1992:231300 Document No. 116:231300 Models of the actin monomer and filament

from fluorescence resonance-energy transfer. O'Donoghue, Sean I.; Hambly,

Brett D.; Dos Remedios, Cristobal G. (Dep. Anat., Univ. Sydney, Sydney, 2006, Australia). Eur. J. Biochem., 205(2), 591-601 (English) 1992. CODEN: EJBCAI. ISSN: 0014-2956.

AB Algorithms were developed for combining fluorescence resonance-energy transfer (**FRET**) efficiency measurements into structural models which predict the relative positions of the chem. groups used in **FRET**. These algorithms were used to construct models of the actin monomer and filament derived solely from **FRET** measurements based on seven distinct loci. A mirror-image pair of monomer models best fit the **FRET** data. One of these models agrees well with the at.-resoln. crystal structure recently published by W. Kabsch et al. (1990). The root-mean-square deviation between this **FRET** model and the crystal structure was about 0.9 nm. Other macromol. models assembled from **FRET** measurements are likely to have a similar resoln. The largest discrepancy was for the Cys10 locus which deviated 1.44 nm from the crystal position. The limitations of the **FRET** method that may have contributed to this discrepancy are discussed. It is concluded that the Cys10 **FRET** data have probably located Cys10 incorrectly in the **FRET** monomer model. Using the **FRET** monomer models, three orientations were found in the filament which best fit the intermonomer **FRET** data. These orientations differ substantially from the at.-resoln. filament model proposed by K. Holmes et al. (1990), largely because of the discrepancies in the Cys10 data. These data should probably be excluded from the anal.;

however, this would leave too few measurements to assemble a filament model. In the near future, addnl. **FRET** measurements to other actin loci may be obtained, so that the filament modeling can be done without the Cys10 data.

L10 ANSWER 37 OF 39 BIOSIS COPYRIGHT 2001 BIOSIS
1992:277835 Document No.: BA94:2485. FLUORESCENCE RESONANCE ENERGY TRANSFER MEASUREMENTS ON CELL SURFACES A SPECTROSCOPIC TOOL FOR DETERMINING PROTEIN

INTERACTIONS. MATYUS L. DEP. BIOPHYSICS, UNIV. MED. SCH. DEBRECEN, P.O. BOX 3, H-4012 DEBRECEN, HUNGARY.. J PHOTOCHEM PHOTOBIO B BIOL, (1992) 12 (4), 323-337. CODEN: JPPBEG. ISSN: 1011-1344. Language: English.

AB The interaction of cell surface components may influence several events during the process of transmembrane signalling. Receptor clustering, conformational changes and altered molecular interactions often play

essential roles in the final outcome of ligand receptor interactions. Fluorescence resonance energy transfer (**FRET**) is an excellent tool which can be used to determine distance relationships and supramolecular structure on cell surfaces. This paper reviews the theoretical basis of fluorescence resonance energy transfer, its spectrofluorometric and flow cytometric applications, and provides a critical evaluation of the **methods**. Finally, examples are given to illustrate the use of the **method of fluorescence energy transfer** in solving biological problems.

L10 ANSWER 38 OF 39 CAPLUS COPYRIGHT 2001 ACS

1991:531318 Document No. 115:131318 On the relationship between distance information derived from cross-linking and from resonance energy transfer,

with specific reference to sites located on myosin heads. Chantler, Peter

D.; Tao, Terence; Stafford, Walter F., III (Dep. Anat. Neurosci., Med. Coll. Pennsylvania, Philadelphia, PA, 19129, USA). Biophys. J., 59(6), 1242-50 (English) 1991. CODEN: BIOJAU. ISSN: 0006-3495.

AB Fluorescence resonance energy transfer (**FRET**) and crosslinking can provide complementary information concerning the relative sepn. of a pair of sites. Crosslinking expts. provide an assessment of the distance of closest approach between a pair of sites. **FRET** measurements, by contrast, yield information about the av. distance between the pair of sites. Hybrid myosins were used to understand the relation between distances obtained for a pair of equiv. sites, one on each myosin head, using both **FRET** (steady-state and time-decay) and crosslinking techniques. The rigid crosslinker, 4,4'-dimaleimidylstilbene-2,2'-disulfonic acid (DMSDS), can efficiently crosslink the 2 myosin

regulatory

light chains, each at residue Cys50 of the Mercenaria regulatory light chain (Chantler, P. D.; Bower, S. M., 1988), indicating that these sites can come within 18 .ANG. of each other. Steady-state and time-decay measurements using fluorescence donor/acceptor pairs located at these

same

sites indicate transfer efficiencies of somewhat <20%, suggesting an av. sepn. of >50 .ANG. between sites (Chantler, P. D.; Tao, T., 1986). Here, are presented theor. calcns. that show that efficient crosslinking can be achieved readily in dynamic systems such as the heads of myosin, even though the necessary subpopulation of proximate mols. at any instant may be below the detection limits of time-decay-**FRET**. Crosslinking expts. can provide important ancillary information about the extent of motions within a macromol. system when used in conjunction with **FRET**. Demonstration of extensive crosslinking does not necessarily indicate a static proximity; the mean sepn. distance should

be

ascertained by other **methods** such as **FRET**.

L10 ANSWER 39 OF 39 CAPLUS COPYRIGHT 2001 ACS

1992:124187 Document No. 116:124187 Structural interpretation of fluorescence resonance-energy transfer measurements. O'Donoghue, Sean I. (Dep. Anat., Univ. Sydney, 2006, Australia). Comput. Appl. Biosci., 7(4),

471-7 (English) 1991. CODEN: COABER. ISSN: 0266-7061.

AB Fluorescence resonance-energy transfer (**FRET**) has been widely used to det. distance information in macromol. systems. However, little has been written about **methods** for combining **FRET** distances into coherent structural models. The author argue that the

methods used so far are inappropriate. This paper describes an algorithm specifically tailored for finding structures from **FRET** measurements. This algorithm finds structures which fit the exptl. measured parameter, the efficiency of energy transfer, rather than derived distances. The algorithm was implemented in Mathematica and applied to **FRET** distances obtained from the contractile protein actin. The approach used is applicable to other exptl. techniques which measure distances between a relatively small no. of loci.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 18:10:32 ON 14 MAY 2001

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L1      8818504 S METHOD
L2      898 S L1 AND FLUORESCENCE ENERGY TRANSFER
L3      62 S L2 AND FRET
L4      0 S L3 AND PHOX
L5      162 S L2 AND PROBE
L6      0 S L5 AND BIODIPYL
L7      13 S L3 AND ASSAY
L8      11 DUP REMOVE L7 (2 DUPLICATES REMOVED)
L9      0 S L3 AND PHOX-BODIPY FL
L10     39 DUP REMOVE L3 (23 DUPLICATES REMOVED)
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=> s l2 and (farinas j?/au)

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L11      0 L2 AND (FARINAS J?/AU)
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=> s farinsa j?/au

```
L12      0 FARINSA J?/AU
```

=> s succinimidyl ester

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L13      1356 SUCCINIMIDYL ESTER
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=> s l13 and aminopentane

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L14      0 L13 AND AMINOPENTANE
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Executing the logoff script...

=> LOG H

COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	115.48	115.63
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE	TOTAL

CA SUBSCRIBER PRICE

ENTRY	SESSION
-7.64	-7.64

SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 18:21:30 ON 14 MAY 2001

L6 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1999:659579 HCAPLUS
 DOCUMENT NUMBER: 131:283619
 TITLE: Methods and reagents for targeting organic compounds
 to selected cellular locations
 INVENTOR(S): Farinas, Javier
 PATENT ASSIGNEE(S): The Regents of the University of California, USA
 SOURCE: PCT Int. Appl., 69 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 INT. PATENT CLASSIF.:
 MAIN: G01N033-53
 SECONDARY: G01N033-567; C07K016-00
 CLASSIFICATION: 9-5 (Biochemical Methods)
 Section cross-reference(s): 1, 3, 6, 15
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9951986	A1	19991014	WO 1999-US7847	19990408 <--
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 9934874	A1	19991025	AU 1999-34874	19990408
PRIORITY APPLN. INFO.:			US 1998-81118	P 19980408
			US 1998-81340	P 19980409
			WO 1999-US7847	W 19990408

ABSTRACT:

The present invention provides methods and reagents for targeting probes to selected cellular locations, through the expression of specific binding partners to that probe within the cell. In one embodiment, the probes may comprise spectroscopic probes that can be used in a method for localizing a specific binding partner within a cell, and for creating assays for post-translational activities. The invention allows the monitoring of the location of such intracellular specific binding partners over time and in response to stimuli, such as test chems. The spectroscopic probes can be used for screening a test chem. for activity. The present invention also includes cells and transgenic organisms comprising the intracellular specific binding partner, wherein the specific binding partner can bind with the spectroscopic probe/ligand conjugate. CHO cells were transfected with cDNAs encoding single chain antibody (sFv) fusion products with a Golgi-targeting human .beta.-1,4-galactosyltransferase fragment. The sFv bound to hapten 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (phOx)-fluorescein conjugate. The Golgi-targeted phOx-fluorescein was used to detect continuous changes in luminal pH in individual cells.

SUPPL. TERM: cell targeting probe expression binding partner;
 spectroscopic probe targeting compd screening;
 posttranslational processing assay spectroscopic probe
 targeting; recombinant single chain antibody cell targeting
 probe; fluorescein hapten conjugate Golgi targeting
 INDEX TERM: Animal cell line

(CHO; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Cytometry
(FACS (fluorescence-activated cell sorting); methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Imaging
(NMR; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Escherichia coli

Yeast
(as host cell; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Proteins, general, reactions
ROLE: RCT (Reactant)
(binding or phosphorylation of, detection of; methods and reagents for targeting org. compds. to selected cellular locations)

and

INDEX TERM: Analysis
(biochem.; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Ligands
ROLE: ARG (Analytical reagent use); BPR (Biological process); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(conjugates with probe; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Post-translational processing
Protein degradation
(detection of; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Prokaryote
(expression vector for; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Proteins, specific or class
ROLE: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BPR (Biological process); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(fluorescent, fusion protein with single chain antibody and with protein of interest; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Chimeric gene
ROLE: BPN (Biosynthetic preparation); BPR (Biological process); BIOL (Biological study); PREP (Preparation); PROC (Process)
(for single chain antibody-protein of interest-fluorescent protein fusion product; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: DNA sequences
Protein sequences
(for single chain antibody; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: pH
(in Golgi app., Golgi-targeted pHx hapten-fluorescein conjugate probing of; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Biological transport

(intracellular; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Animal cell;
(mammalian; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Drug screening
Drug targeting
Eukaryote (Eukaryotae)
Fluorescence
Fluorescent probes
Genetic vectors
Positron-emission tomography
Spectroscopy
(methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Phosphorylation, biological
(of proteins, detection of; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Molecular cloning
(of single chain antibody-protein of interest fusion products; methods and reagents for targeting org. compds. to selected cellular locations)

compds.

INDEX TERM: Plasmids
(pHook, in plasma membrane targeting of single chain antibody; methods and reagents for targeting org. compds. to selected cellular locations)

compds.

INDEX TERM: Genetic element
Promoter (genetic element)
ROLE: BPN (Biosynthetic preparation); BPR (Biological process); BIOL (Biological study); PREP (Preparation); PROC (Process)
(response element, operably linked with nucleic acid encoding single chain antibody; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Genetic element
ROLE: BPN (Biosynthetic preparation); BPR (Biological process); BIOL (Biological study); PREP (Preparation); PROC (Process)
(signal sequence, for preprolactin, for targeting endoplasmic reticulum; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Membrane, biological
(single chain antibody bound to and probe-ligand conjugate permeable through; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Fluorescent substances
(single chain antibody with second binding site for; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Cell
(single chain antibody-expressing, probe localization in;
methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Antibodies
ROLE: ARG (Analytical reagent use); BOC (Biological occurrence); BPN (Biosynthetic preparation); BPR (Biological

process); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); PROC (Process); USES (Uses)
(single chain, cell expressing, probe localization in; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Cell membrane
Endoplasmic reticulum
(targeting of; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Golgi apparatus
(targeting of; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Mouse
(transgenic, expressing single chain antibody; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: Fusion proteins (chimeric proteins)
ROLE: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BPR (Biological process); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(with single-chain antibodies; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 88899-55-2, Bafilomycin A1
ROLE: BAC (Biological activity or effector, except adverse);
BIOL (Biological study)
(Golgi-targeted phOx hapten-fluorescein conjugate
of Golgi pH response to; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 9054-94-8P
ROLE: BPN (Biosynthetic preparation); BPR (Biological process); BIOL (Biological study); PREP (Preparation); PROC (Process)
(N-terminal fragment of, for trans-Golgi targeting; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 246535-34-2P
ROLE: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BPR (Biological process); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(amino acid sequence; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 246256-51-9
ROLE: ARG (Analytical reagent use); BOC (Biological occurrence); BPR (Biological process); PRP (Properties); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)
(as hapten-fluorophore conjugate; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 246149-19-9P 246252-35-7P 246256-49-5P
ROLE: ARG (Analytical reagent use); BOC (Biological occurrence); BPR (Biological process); PRP (Properties); SPN
(Synthetic preparation); ANST (Analytical study); BIOL

(Biological study); OCCU (Occurrence); PREP (Preparation); PROC (Process); USES (Uses)
 (as hapten-fluorophore conjugate; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 246149-20-2P
 ROLE: ARG (Analytical reagent use); BPR (Biological process); SPN (Synthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
 (as hapten-fluorophore conjugate; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 246149-15-5P 246149-17-7P
 ROLE: ARG (Analytical reagent use); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation); USES (Uses)
 (as hapten-fluorophore conjugate; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 141-43-5, Ethanolamine, reactions 462-94-2, 1,5-Diaminopentane 2615-25-0, trans-1,4-Diaminocyclohexane
 15646-46-5 187328-05-0 146616-66-2 246149-18-8 246256-50-8
 ROLE: RCT (Reactant)
 (in synthesis of hapten-fluorophore conjugates; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 246149-16-6P
 ROLE: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation)
 (in synthesis of hapten-fluorophore conjugates; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 246535-35-3P
 ROLE: BPN (Biosynthetic preparation); BPR (Biological process); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)
 (nucleotide sequence; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 65637-74-3P, Prolactin
 ROLE: BPN (Biosynthetic preparation); BPR (Biological process); BIOL (Biological study); PREP (Preparation); PROC (Process)
 (signal sequence for, for targeting endoplasmic reticulum; methods and reagents for targeting org. compds. to selected cellular locations)

INDEX TERM: 246535-41-1, PN: WO9951986 SEQID: 2 unclaimed DNA
 246535-42-2, PN: WO9951986 SEQID: 3 unclaimed DNA
 ROLE: PRP (Properties)
 (unclaimed nucleotide sequence; methods and reagents for targeting org. compds. to selected cellular locations)

REFERENCE COUNT: 7
 REFERENCE(S):
 (1) Mori; US 5703369 A 1997
 (2) Pastan; US 5328984 A 1994 HCAPLUS
 (3) Pastan; US 5602095 A 1997 HCAPLUS
 (4) Redington; US 4618827 A 1986
 (5) Richardson; Proc Natl Acad Sci USA 1995, V92, P3137 HCAPLUS

- (6) Vold; US 5561049 A 1996 HCAPLUS
- (7) Yuan; Biochem J 1996, V318, P591 HCAPLUS

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(FILE 'HOME' ENTERED AT 17:58:30 ON 25 MAY 2001)

FILE 'CAOLD, CAPLUS, CROPU, DGENE, DPCI, ENCOMPPAT, ENCOMPPAT2, EUROPATFULL, IFIPAT, INPADOC, JAPIO, PAPERCHEM2, PATDD, PATDPA, PATOSDE, PATOSEP, PATOSWO, PCTFULL, PIRA, RAPRA, SYNTHLINE, TULSA, TULSA2, USPATFULL, WPIDS' ENTERED AT 17:58:36 ON 25 MAY 2001

FILE 'HCAPLUS' ENTERED AT 17:59:35 ON 25 MAY 2001

L1 2677 S (SINGLE CHAIN AND (ANTIBOD# OR ANTIBODIES)) OR (ANTIBODIES
(L
L2 1897846 S CELL#
L3 1589 S L1 AND L2
L4 5 S L3 AND LOCAL? AND PROBE#
L5 32 S L3 AND PROBE#
L6 16 S L5 AND PY<=1998
L7 16 S L5 AND PD<=1998

FILE 'HCAPLUS' ENTERED AT 18:23:00 ON 25 MAY 2001

E INTRABODIES/CT
E SINGLE CHAIN ANTIBODIES/CT
E E3+ALL
E ANTIBODIES (L) SINGLE CHAIN/CT
E E3+ALL
L8 2899 S (SINGLE CHAIN AND (ANTIBOD# OR ANTIBODIES)) OR (ANTIBODIES
(L
L9 1740 S L8 AND CELL?
L10 36 S L9 AND PROBE#
L11 16 S L10 AND PD<19980408

FILE 'CAOLD, CAPLUS, CROPU, DGENE, DPCI, ENCOMPPAT, ENCOMPPAT2, EUROPATFULL, IFIPAT, INPADOC, JAPIO, PAPERCHEM2, PATDD, PATDPA, PATOSDE, PATOSEP, PATOSWO, PCTFULL, PIRA, RAPRA, SYNTHLINE, TULSA, TULSA2, USPATFULL, WPIDS' ENTERED AT 18:30:17 ON 25 MAY 2001

L12 23361 S L8
L13 18913 S L12 AND CELL#
L14 16013 S L13 AND EXPRESS?
L15 9448 S L14 AND PROBE#
L16 8579 S L15 AND MEMBRANE#
L17 6206 S L16 AND (LIGAND# OR (LIGANDS (L) CONJUGATED))
L18 4920 S L17 AND LOCAL?
L19 405 S L18 AND ((FLUORESCENT PROBE#) OR (FLUORESCENT SUBSTANCE#) OR
L20 404 DUP REM L19 (1 DUPLICATE REMOVED)
L21 398 S L20 AND (LINK# OR LINKER# OR COUPLING AGENT# OR LINKING AGEN
L22 111 S L21 AND POSITRON EMISSION TOMOGRAPHY
L23 0 S L22 AND NMR AND FLUORESCENCE ANISOTROPY
L24 102 S L22 AND NMR
L25 0 S L24 AND ANISOTROPY
L26 0 S L24 AND PY<=1998
L27 27 S L19 AND FLUORESCENCE EMISSION
L28 3 S L27 AND PY<=1998

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(FILE 'HOME' ENTERED AT 21:56:56 ON 25 MAY 2001)

FILE 'HCAPLUS' ENTERED AT 21:57:25 ON 25 MAY 2001

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E CELL LOCALIZATION/CT
E CELL TARGETING/CT
E BIOLOGICAL TRANSPORT/CT
E E3+ALL
E POSITRON EMISSION TOMOGRAPHY/CT
E E12+ALL
E POSITRON-EMISSION TOMOGRAPHY/CT
E E3+ALL
E FLUORESCENCE ANISOTROPY/CT
E ANISOTROPY/CT
E ANISOTROPY
E ANISOTROPY/CT
E E3+ALL
E FLUORESCENCE ANISOTROPY/CT
E FLUORESCENCE ANISOTROPY/CT
E FLUORESCENCE/CT
E FLUORESCENCE/CT
E E3+ALL
E FLUORESCENCE EMISSION/CT
L1      160037 S BIOLOGICAL TRANSPORT OR CELL TARGET? OR CELL LOCAL?
L2      2899 S (SINGLE CHAIN AND (ANTIBOD# OR ANTIBODIES)) OR (ANTIBODIES
(L
L3      79 S L1 AND L2
L4      2 S L3 AND PROBE#
L5      45 S L3 AND PD<19980408
E WO9951986/PN 25
L6      1 S E3
L7      5211 S POSITRON EMISSION TOMOGRAPHY OR (RADIOGRAPHY (L) LAMINOG.,
PO
L8      1 S L7 AND L3
SET SMA OFF
SEL RAN.HCAPLUS(5) L8 1
SET SMA LOGIN
L9      1 S E1
L10     113 S L1 AND L7
L11     73 S L10 AND PD<19980408
L12     0 S L11 AND (ANTIBODIES OR ANTIBODY)
L13     4 S L11 AND PROBE#
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L13 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:450216 HCAPLUS

DOCUMENT NUMBER: 121:50216

TITLE: Effect of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin on in vivo L-[.beta.-11C]DOPA turnover in the rat striatum with infusion of L-tyrosine

AUTHOR(S): Tsukada, H.; Lindner, K. -J.; Hartvig, P.; Tani, Y.; Bjurling, P.; Kihlberg, T.; Westerberg, G.; Watanabe, Y.; Laangstroem, B.

CORPORATE SOURCE: Cent. Res. Lab. PET Cent., Hamamatsu Photonics K. K., Shizuoka, Japan

SOURCE: J. Neural Transm.: Gen. Sect. (1994), 95(1), 1-15

CODEN: JNGSE8; ISSN: 0300-9564

DOCUMENT TYPE: Journal

LANGUAGE: English

AB L-[11C]DOPA, combined with **positron emission tomog.** (PET), has made possible the assessment of dopamine turnover in vivo. Before the evaluation of PET study with L-[11C]DOPA in the primate, the effect of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin (6R-BH4) and/or L-tyrosine infusion on L-[11C]DOPA turnover was analyzed in the rat striatal tissue and in the striatal extracellular fluid using microdialysis. L-[11C]DOPA was rapidly taken up into the brain after

i.v. injection and converted to [11C]dopamine, [11C]DOPAC and [11C]homovanillic

acid (HVA) in the striatal tissue. Small amt. of 3-O-methyl-[11C]DOPA, a product of DOPA by 3-O-methylation in peripheral tissues, was also detected in the striatal tissue. The striatum/cerebellum ratio of total radioactivity uptake was linear against time up to 40 min after L-[11C]DOPA injection. The uptake ratio, increased by 6R-BH4 administration, was further increased by L-tyrosine infusion. The in

vivo microdialysis technique was further applied to det. L-[11C]DOPA and its metabolites in striatal extracellular fluid (ECF). The peripheral administration of 6R-BH4 (50mg/kg) induced elevation of [11C]DOPA concn. in ECF in the early phase after injection, following higher radioactivity in [11C]dopamine and [11C]HVA fractions than those in control animals at late phase. The 6R-BH4-induced elevation of [11C]DOPA uptake and the radioactivity of its metabolites was further enhanced by the continuous infusion of L-tyrosine at a dose of 1.0 .mu.mol/min/kg. L-Tyrosine infusion alone did not induce the elevation of radioactivity. The

results suggest that [11C]DOPA might be a useful **probe** to evaluate the effect of 6R-BH4 and/or L-tyrosine loading in the primate.

SO J. Neural Transm.: Gen. Sect. (1994), 95(1), 1-15
CODEN: JNGSE8; ISSN: 0300-9564

AB L-[11C]DOPA, combined with **positron emission tomog.** (PET), has made possible the assessment of dopamine turnover in vivo. Before the evaluation of PET study with L-[11C]DOPA in the primate, the effect of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin (6R-BH4) and/or L-tyrosine infusion on L-[11C]DOPA turnover was analyzed in the rat striatal tissue and in the striatal extracellular fluid using microdialysis. L-[11C]DOPA was rapidly taken up into the brain after

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product of DOPA by 3-O-methylation in peripheral tissues, was also detected in the striatal tissue. The striatum/cerebellum ratio of total radioactivity uptake was linear against time up to 40 min after L-[11C]DOPA injection. The uptake ratio, increased by 6R-BH4 administration, was further increased by L-tyrosine infusion. The in vivo microdialysis technique was further applied to det. L-[11C]DOPA and its metabolites in striatal extracellular fluid (ECF). The peripheral administration of 6R-BH4 (50mg/kg) induced elevation of [11C]DOPA concn. in ECF in the early phase after injection, following higher radioactivity in [11C]dopamine and [11C]HVA fractions than those in control animals at late phase. The 6R-BH4-induced elevation of [11C]DOPA uptake and the radioactivity of its metabolites was further enhanced by the continuous infusion of L-tyrosine at a dose of 1.0 .mu.mol/min/kg. L-Tyrosine infusion alone did not induce the elevation of radioactivity. The results suggest that [11C]DOPA might be a useful **probe** to evaluate the effect of 6R-BH4 and/or L-tyrosine loading in the primate.

IT **Biological transport**
(absorption, of DOPA, in cerebellum and striatum, **positron emission tomog.** assessment of, tetrahydrobiopterin and tyrosine effect on)

IT **Tomography**
(**positron-emission**, in dopamine turnover measurement, in striatum with [11C]DOPA, tetrahydrobiopterin and tyrosine effect on)

IT Brain, metabolism
(striatum, dopamine turnover, [11C]DOPA and **positron emission tomog.** in assessment of, tetrahydrobiopterin and tyrosine effect on)

IT 62989-33-7 60-18-4, L-Tyrosine, biological studies
RL: ANST (Analytical study)
(dopamine turnover in response to, in striatum, carbon-11-labeled DOPA and **positron emission tomog.** in assessment of)

L13 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1992:527656 HCAPLUS

DOCUMENT NUMBER: 117:127656

TITLE: Membrane trapping of carbon-11-labeled 1,2-diacylglycerols as a basic concept for assessing phosphatidylinositol turnover in neurotransmission process

AUTHOR(S): Imahori, Yoshio; Fujii, Ryou; Ueda, Satoshi; Matsumoto, Keigo; Wakita, Kazuo; Ido, Tatsuo; Nariai, Tadashi; Nakahashi, Hisamitsu

CORPORATE SOURCE: Dep. Neurosurg., Kyoto Prefect. Univ. Med., Kyoto, Japan

SOURCE: J. Nucl. Med. (1992), 33(3), 413-22
CODEN: JNMEAQ; ISSN: 0161-5505

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The uptake mechanism of 1,2-[11C]diacylglycerols (DAG) was studied and its

use as a **probe** for the measurement of phosphatidylinositol (PI) turnover was verified. A method of synthesis for producing rac-1,2-[11C]DAG using [11C]ethylketene was developed to label the 1- of 3-hydroxyl group of 2-monoacylglycerol. After i.v. injection, these tracers were metabolized rapidly in the rat brain cortex to phosphatidic acids, phosphatidylinositols, and phosphatidylinositol phosphates. The

brain cortex anesthetized by barbiturate, which represents inhibited state of synaptic transmission, did not produce differences in uptake values between sn-1,2-[11C]DAG and rac-1,2-[11C]DAG. However, in the liver, lung, and pancreas under the same conditions, the uptake values of rac-1,2-[11C]DAG were higher than those of sn-1,2-[11C]DAG, in which the labeling position was on the 2-hydroxyl group in the sn type. These findings suggest that the lipase activity in the brain should be disregarded because lipase predominantly hydrolyzes the 1- or 3-position of rac-1,2-[11C]DAG, which should be the main factor producing the differences in uptake values in other organs. Cholinergic stimulation prompted accumulation of 1,2-[11C]DAG in the conscious rat brain. In conclusion, sn-1,2-[11C]DAG, administered even in the racemic mixt.,

could serve as a tracer that becomes mixed with receptor-linked PI turnover and could accumulate in the brain based on the membrane trapping mechanism.

SO J. Nucl. Med. (1992), 33(3), 413-22

CODEN: JNMEAQ; ISSN: 0161-5505

AB The uptake mechanism of 1,2-[11C]diacylglycerols (DAG) was studied and its

use as a **probe** for the measurement of phosphatidylinositol (PI) turnover was verified. A method of synthesis for producing rac-1,2-[11C]DAG using [11C]ethylketene was developed to label the 1- of 3-hydroxyl group of 2-monoacylglycerol. After i.v. injection, these tracers were metabolized rapidly in the rat brain cortex to phosphatidic acids, phosphatidylinositols, and phosphatidylinositol phosphates. The brain cortex anesthetized by barbiturate, which represents inhibited

state of synaptic transmission, did not produce differences in uptake values between sn-1,2-[11C]DAG and rac-1,2-[11C]DAG. However, in the liver, lung, and pancreas under the same conditions, the uptake values of rac-1,2-[11C]DAG were higher than those of sn-1,2-[11C]DAG, in which the labeling position was on the 2-hydroxyl group in the sn type. These findings suggest that the lipase activity in the brain should be disregarded because lipase predominantly hydrolyzes the 1- or 3-position of rac-1,2-[11C]DAG, which should be the main factor producing the differences in uptake values in other organs. Cholinergic stimulation prompted accumulation of 1,2-[11C]DAG in the conscious rat brain. In conclusion, sn-1,2-[11C]DAG, administered even in the racemic mixt.,

could serve as a tracer that becomes mixed with receptor-linked PI turnover and could accumulate in the brain based on the membrane trapping mechanism.

ST brain phosphatidylinositol turnover detn neurotransmission; membrane carbon 11 diglyceride detn PET; **positron emission tomog** diglyceride brain

IT **Biological transport**

(absorption, of carbon-11-labeled diglycerides, phosphatidylinositol turnover in neurotransmission in relation to)

L13 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1992:79478 HCAPLUS

DOCUMENT NUMBER: 116:79478

TITLE: 11C-aminocyclohexane carboxylic acid - a **probe** for measurement of amino acid transport into brain

AUTHOR(S): Shulkin, B.; Koeppe, R.; Allen, R.; Kollros, P.; Price, J.; Betz, L.; Mangner, T.; Rosenspire, K.; Kuhl, D.; Agranoff, B.

CORPORATE SOURCE: Dep. Med., Univ. Michigan, Ann Arbor, MI, USA

SOURCE: Nuklearmedizin, Suppl. (Stuttgart) (1990), 26(Nucl. Med.), 80-3

CODEN: NMBSAG; ISSN: 0550-3175

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The purpose of this study is to assess the potential utility of 11C-ACHC (aminocyclohexenecarboxylic acid) as a **probe** for the measurement of regional amino acid transport into brain using dynamic PET (**positron emission tomog.**) scanning and tracer kinetic modeling.

TI 11C-aminocyclohexane carboxylic acid - a **probe** for measurement of amino acid transport into brain

SO Nuklearmedizin, Suppl. (Stuttgart) (1990), 26(Nucl. Med.), 80-3
CODEN: NMBSAG; ISSN: 0550-3175

AB The purpose of this study is to assess the potential utility of 11C-ACHC (aminocyclohexenecarboxylic acid) as a **probe** for the measurement of regional amino acid transport into brain using dynamic PET (**positron emission tomog.**) scanning and tracer kinetic modeling.

ST carbon 11 aminocyclohexanecarboxylate **positron emission tomog**; amino acid transport brain **tomog**;
cyclohexanecarboxylate carbon 11 **positron emission tomog**

IT Brain, metabolism
(amino acids transport in human, **positron emission tomog.** of, with carbon-11-aminocyclohexane carboxylic acid)

IT **Biological transport**
(of amino acids, in brain of human, position emission tomog. of)

IT Amino acids, biological studies
RL: BIOL (Biological study)
(transport of, in human brain, **positron emission tomog.** of)

IT **Tomography**
(**positron-emission**, of amino acid transport in brain of human, with carbon-11-aminocyclohexane carboxylic acid)

IT 138746-38-0
RL: BIOL (Biological study)
(**positron emission tomog.** with, of amino acid transport in brain of human)

L13 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1988:469822 HCAPLUS

DOCUMENT NUMBER: 109:69822

TITLE: Assessment of [11C]-L-methionine transport into the human brain

AUTHOR(S): O'Tuama, Lorcan A.; Guilarte, Tomas R.; Douglass, Kenneth H.; Wagner, Henry N., Jr.; Wong, Dean F.; Dannals, Robert F.; Ravert, H. T.; Wilson, A. A.; LaFrance, Norman D.; et al.

CORPORATE SOURCE: Div. Nucl. Med., Johns Hopkins Med. Inst., Baltimore, MD, 21205-2179, USA

SOURCE: J. Cereb. Blood Flow Metab. (1988), 8(3), 341-5

CODEN: JCBMDN; ISSN: 0271-678X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Neutral amino acid transport into human brain was measured using a dual-**probe** positron detection system or **positron emission tomog.** (PET). Rate consts. (mL/min/cm³) for brain accumulation of L-[11C]methionine measured with the dual detector ranged 0.012-0.078 (av. 0.031) under baseline conditions and 0.010-0.017 (av. 0.014) after administration of nonradioactive L-phenylalanine (100

mg/kg). The net rate of brain accumulation of L-methionine ranged 0.42-2.89 (av. 1.28) nmol/min/cm³, and decreased by 27.5-91.2% (av. 53.9%) after L-phenylalanine. PET-estd. accumulation rates (mL/min/cm³) of L-[11C]methionine ranged 0.004-0.028 (av. 0.016) baseline and 0.010-0.021 (av. 0.017) after L-phenylalanine. Initial vols. of distribution (mL/cm³) of L-[11C]methionine (dual detector) were 0.044-0.070 (av. 0.058) baseline and 0.032-0.074 (av. 0.051) after phenylalanine and (PET) 0.026-0.098 (av. 0.051) baseline and 0.021-0.061 (av. 0.042) after phenylalanine. PET permits more accurate measurement of tracer accumulation by brain, excluding noncerebral regions included in dual-detector measurements.

The dual-detector system permits better temporal resoln., facilitating kinetic anal., and requires only 0.025% the dose of tracer needed for PET. Multiple studies in the same patient are thus possible at low cost.

SO J. Cereb. Blood Flow Metab. (1988), 8(3), 341-5
CODEN: JCBMDN; ISSN: 0271-678X

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The dual-detector system permits better temporal resoln., facilitating kinetic anal., and requires only 0.025% the dose of tracer needed for PET. Multiple studies in the same patient are thus possible at low cost.

ST **positron emission tomog** methionine brain;
methionine transport detn brain; neutral amino acid transport brain;
carbon 11 methionine transport brain

IT Brain, metabolism
(methionine transport by human, dual-**probe** positron detection or **positron emission tomog.** in detn. of)

IT Amino acids, biological studies
RL: BIOL (Biological study)
(transport of, in brain of human, dual-**probe** positron detection or **positron emission tomog.** in study of)

IT **Biological transport**
(absorption, of methionine, by human brain, dual-**probe** positron detection or **positron emission**

tomog. in detn. of)

IT Tomography
(positron-emission, of methionine transport, in
brain of human with carbon-11-labeled methionine)

IT 60305-58-0
RL: ANST (Analytical study)
(positron emission tomog. with, of
methionine transport in brain of human)

IT 63-68-3, Methionine, biological studies
RL: BIOL (Biological study)
(transport of, in brain of human, dual-probe positron
detection or positron emission tomog. in
study of)

FILE 'HCAPLUS' ENTERED AT 22:09:36 ON 23 MAY 2001

L1 2166 S (SINGLE CHAIN (W) (ANTIBOD# OR ANTIBODIES)) OR (ANTIBODIES
(L)
L2 280250 S LIGAND# OR (LIGANDS (L) CONJUGATED)
L3 81447 S LINK# OR LINKER# OR COUPLING AGENT# OR LINKING AGENT#
L4 214288 S PROBE#
L5 0 S L1 AND L2 AND L3 AND L4
L6 3 S L1 AND L2 AND L4
L7 1 S L6 AND PD<19980408
L8 203 S L1 AND L2
L9 22 S L8 AND L3
L10 15 S L9 AND PD<19980408

=> d ibib hit 1

L10 ANSWER 1 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:163604 HCAPLUS

DOCUMENT NUMBER: 128:226232

TITLE: Cobalt complex bioconjugates, preparation thereof,
and

delivery of bioactive agents
INVENTOR(S): Grissom, Charles B.; West, Frederick G.; Howard, W.
Allen, Jr.

PATENT ASSIGNEE(S): University of Utah Research Foundation, USA; Grissom,
Charles B.; West, Frederick G.; Howard, W. Allen, Jr.

SOURCE: PCT Int. Appl., 91 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9808859	A1	19980305	WO 1997-US14140	19970822 <--
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9741482	A1	19980319	AU 1997-41482	19970822 <--
EP 1007533	A1	20000614	EP 1997-939382	19970822
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
JP 2001501596	T2	20010206	JP 1998-511674	19970822
PRIORITY APPLN. INFO.:			US 1996-24430	P 19960827
			US 1996-25036	P 19960827
			WO 1997-US14140	W 19970822

OTHER SOURCE(S): MARPAT 128:226232

PI WO 9808859 A1 19980305

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9808859	A1	19980305	WO 1997-US14140	19970822 <--
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9741482	A1	19980319	AU 1997-41482	19970822 <--
EP 1007533	A1	20000614	EP 1997-939382	19970822
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
JP 2001501596	T2	20010206	JP 1998-511674	19970822

IT **Single chain antibodies**

RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(ScFv, targeting mol.; cobalt complex bioconjugates, prepn., and

delivery of bioactive agents)

IT **Ligands**
 RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
 (exchange; cobalt complex bioconjugates, prepn., and delivery of
 bioactive agents)

IT **Coupling agents**
 (self-destructing; cobalt complex bioconjugates, prepn., and delivery
 of bioactive agents)

=> d ibib ab hit 1

L10 ANSWER 1 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:163604 HCAPLUS

DOCUMENT NUMBER: 128:226232

TITLE: Cobalt complex bioconjugates, preparation thereof,
 and

delivery of bioactive agents

INVENTOR(S): Grissom, Charles B.; West, Frederick G.; Howard, W.
 Allen, Jr.

PATENT ASSIGNEE(S): University of Utah Research Foundation, USA; Grissom,
 Charles B.; West, Frederick G.; Howard, W. Allen, Jr.

SOURCE: PCT Int. Appl., 91 pp.
 CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9808859	A1	19980305	WO 1997-US14140	19970822 <--
W:			AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM	
RW:			GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG	
AU 9741482	A1	19980319	AU 1997-41482	19970822 <--
EP 1007533	A1	20000614	EP 1997-939382	19970822
R:			AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI	
JP 2001501596	T2	20010206	JP 1998-511674	19970822
PRIORITY APPLN. INFO.:			US 1996-24430 P	19960827
			US 1996-25036 P	19960827
			WO 1997-US14140 W	19970822

OTHER SOURCE(S): MARPAT 128:226232

AB The invention relates to bioconjugates and the delivery of bioactive agents which are preferably targeted for site-specific release in cells, tissues or organs. More particularly, the invention relates to bioconjugates which comprise of bioactive agent and an organocobalt complex. The bioactive agent is covalently bonded directly or indirectly to the cobalt atom of the organocobalt complex. The bioactive agent is released from the bioconjugate by the cleavage of the covalent bond between the bioactive agent and the cobalt atom in the organocobalt complex. The cleavage may occur as a result of normal displacement by cellular nucleophiles or enzymic action, but is preferably caused to

occur

selectively at a predetd. release site by application of an external signal. The external signal may be light or photoexcitation, i.e. photolysis, or it may be ultrasound, i.e. sonolysis. Further, if the photolysis takes places in the presence of a magnetic field surrounding the release site, the release of the bioactive agent into surrounding healthy tissue is minimized. Prepn. and antitumor activity of a chlorambucil-cobalamin bioconjugate are included.

PI WO 9808859 A1 19980305
PATENT NO. KIND DATE APPLICATION NO. DATE

PI WO 9808859 A1 19980305 WO 1997-US14140 19970822 <--
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
AU 9741482 A1 19980319 AU 1997-41482 19970822 <--
EP 1007533 A1 20000614 EP 1997-939382 19970822
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
JP 2001501596 T2 20010206 JP 1998-511674 19970822
IT **Single chain antibodies**
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses) (ScFv, targeting mol.; cobalt complex bioconjugates, prepn., and delivery of bioactive agents)
IT **Ligands**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process) (exchange; cobalt complex bioconjugates, prepn., and delivery of bioactive agents)
IT **Coupling agents**
RL: (self-destructing; cobalt complex bioconjugates, prepn., and delivery of bioactive agents)

=> d ibib ab hit 2

L10 ANSWER 2 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:718994 HCAPLUS

DOCUMENT NUMBER: 128:21612

TITLE: Linear gene fusions of antibody fragments with streptavidin can be linked to biotin labeled

secondary

AUTHOR(S): molecules to form bispecific reagents
Pearce, Lesley A.; Oddie, Geoffrey W.; Coia, Gregory; Kortt, Alexander A.; Hudson, Peter J.; Lilley, Glenn G.

CORPORATE SOURCE: Division of Biomolecular Engineering, CSIRO, Parkville, 3052, Australia

SOURCE: Biochem. Mol. Biol. Int. (1997), 42(6), 1179-1188

CODEN: BMBIES; ISSN: 1039-9712

PUBLISHER: Academic

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Monomeric **single chain antibody** (scFv) fragments lack both the avidity of the bivalent IgG, or (Fab')₂ fragment, and the effector functions conferred by the Fc domain. For certain

diagnostic or therapeutic applications it may be desirable to link these mols. to other proteins, **antibodies**, enzymes or peptide **ligands**, and chem. or recombinant methods have been developed to produce many of these crosslinked reagents. One approach has been to link an antibody fragment to streptavidin which can bind a second biotinylated mol. to create a higher affinity, bifunctional or bispecific mol. To demonstrate the applicability of this technol., an anti-neuraminidase NC10 scFv-streptavidin fusion was expressed in E. coli and the product was refolded and purified to homogeneity from 6 M guanidine hydrochloride. Anal. in a BIAcoreTM biosensor showed that the NC10 scFv moiety reacted with immobilized neuraminidase and that the core streptavidin moiety was able to bind biotinylated anti-ferritin Fab' to produce a new model bispecific reagent which bound ferritin. Conceptually, this design principle can be applied to the creation of useful diagnostic and possibly therapeutic mols.

SO Biochem. Mol. Biol. Int. (1997), 42(6), 1179-1188
CODEN: BMBIES; ISSN: 1039-9712

AB Monomeric **single chain antibody** (scFv) fragments lack both the avidity of the bivalent IgG, or (Fab')₂ fragment, and the effector functions conferred by the Fc domain. For certain diagnostic or therapeutic applications it may be desirable to link these mols. to other proteins, **antibodies**, enzymes or peptide **ligands**, and chem. or recombinant methods have been developed to produce many of these crosslinked reagents. One approach has been to link an antibody fragment to streptavidin which can bind a second biotinylated mol. to create a higher affinity, bifunctional or bispecific mol. To demonstrate the applicability of this technol., an anti-neuraminidase NC10 scFv-streptavidin fusion was expressed in E. coli and the product was refolded and purified to homogeneity from 6 M guanidine hydrochloride. Anal. in a BIAcoreTM biosensor showed that the NC10 scFv moiety reacted with immobilized neuraminidase and that the core streptavidin moiety was able to bind biotinylated anti-ferritin Fab' to produce a new model bispecific reagent which bound ferritin. Conceptually, this design principle can be applied to the creation of useful diagnostic and possibly therapeutic mols.

IT **Single chain antibodies**

RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Fv fragments; linear gene fusions of antibody fragments with streptavidin can be linked to biotin labeled secondary mols. to form bispecific reagents in relation to)

=> d ibib ab hit 3-15

L10 ANSWER 3 OF 15 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:85151 HCAPLUS
DOCUMENT NUMBER: 126:85615
TITLE: Self-associating peptide domains for use in the formation of hetero- or homooligomeric proteins
INVENTOR(S): Pack, Peter; Hoess, Adolf
PATENT ASSIGNEE(S): Morphosys Gesellschaft Fuer Proteinoptimierung MbH, Germany; Pack, Peter; Hoess, Adolf
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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	WO 9637621	A2	19961128	WO 1996-EP2230	19960523 <--
	WO 9637621	A3	19970103		
	W: CA, CN, JP, US				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT,				
SE	CA 2222055	AA	19961128	CA 1996-2222055	19960523 <--
	EP 827544	A2	19980311	EP 1996-916159	19960523 <--
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 11508126	T2	19990721	JP 1996-535396	19960523
PRIORITY APPLN. INFO.:				EP 1995-107914	19950523
				WO 1996-EP2230	19960523

AB Self-assocg. peptides that can be used to direct the oligomerization of proteins into homo- or heterooligomers are described for use in the manuf.

of oligomeric proteins by expression of cloned genes. These peptides do not significantly interfere with secretion, expression yields and the independent folding of functional domains attached to them by flexible protease-resistant **linkers**. Modular gene cassettes encoding functional domains, **linkers** and multimerization domain can easily be combined into a cistron encoding the multimeric protein. Translation in a suitable host results in self-assembly to multimers larger than dimers. In cases in which one or both functional domains are not expressible in sufficient yields or fold into their native forms in the same expression host, multimeric proteins can be produced by manuf.

of

the subunits sep. by, e.g., in vitro translation, peptide synthesis and/or

refolding and subsequently, e.g., chem. coupled to the remaining part of the multimeric protein. The use of these peptides is demonstrated by using them to build a tetramer of a single-chain anti-Ley antibody and a metal-binding domain using a tetramerization peptide derived from p53.

PI	WO 9637621	A2	19961128		
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9637621	A2	19961128	WO 1996-EP2230	19960523 <--
	WO 9637621	A3	19970103		
	W: CA, CN, JP, US				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT,				
SE	CA 2222055	AA	19961128	CA 1996-2222055	19960523 <--
	EP 827544	A2	19980311	EP 1996-916159	19960523 <--
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 11508126	T2	19990721	JP 1996-535396	19960523

AB Self-assocg. peptides that can be used to direct the oligomerization of proteins into homo- or heterooligomers are described for use in the manuf.

of oligomeric proteins by expression of cloned genes. These peptides do not significantly interfere with secretion, expression yields and the independent folding of functional domains attached to them by flexible protease-resistant **linkers**. Modular gene cassettes encoding functional domains, **linkers** and multimerization domain can easily be combined into a cistron encoding the multimeric protein. Translation in a suitable host results in self-assembly to multimers larger than dimers. In cases in which one or both functional domains are not expressible in sufficient yields or fold into their native forms in the same expression host, multimeric proteins can be produced by manuf.

of

the subunits sep. by, e.g., in vitro translation, peptide synthesis and/or refolding and subsequently, e.g., chem. coupled to the remaining part of the multimeric protein. The use of these peptides is demonstrated by using them to build a tetramer of a single-chain anti-Ley antibody and a metal-binding domain using a tetramerization peptide derived from p53.

IT E-selectin
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (ligand 1 for, oligomeric **single-chain antibodies** binding; self-assocg. peptide domains for use in formation of hetero- or homooligomeric proteins)

IT Ley antigen
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (oligomeric **single-chain antibodies** binding; self-assocg. peptide domains for use in formation of hetero- or homooligomeric proteins)

IT Plasmid vectors
 (pMSL5-P53-His, chimeric gene for self-assocg. **single chain antibody** on; self-assocg. peptide domains for use in formation of hetero- or homooligomeric proteins)

IT **Antibodies**
 RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
 (**single-chain**, formation of oligomers of; self-assocg. peptide domains for use in formation of hetero- or homooligomeric proteins)

IT 107-73-3, Phosphocholine
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (oligomeric **single-chain antibodies** binding; self-assocg. peptide domains for use in formation of hetero- or homooligomeric proteins)

L10 ANSWER 4 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:590592 HCAPLUS

DOCUMENT NUMBER: 125:269698

TITLE: Intracellular **single-chain antibody** inhibits integrin VLA-4 maturation and function

AUTHOR(S): Yuan, Qian; Strauch, Kathryn L.; Lobb, Roy R.; Hemler,

Martin E.

CORPORATE SOURCE: Dana-Farber Cancer Inst., Harvard Med. Sch., Boston, MA, 02115, USA

SOURCE: Biochem. J. (1996), 318(2), 591-596

CODEN; BIJOAK; ISSN: 0264-6021

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A **single-chain antibody** construct was prepd. contg. the VH and VL regions of anti-(integrin .alpha.4) antibody HP1/2, an interchain **linker** and a KDEL endoplasmic reticulum retention sequence. Intracellular expression of this **single-chain antibody** caused cell-surface expression of .alpha.4.beta.1 integrin to be decreased by 80% on selected RD cells and by 65-100% on selected Jurkat cells, relative to mock transfectants. Immunopptn. from **single-chain-antibody**-transfected cells showed that the **single-chain antibody** was complexed with the integrin .alpha.4 and .beta.1 subunits, and the diminished sizes of .alpha.4 and .beta.1 were consistent with impaired maturation. Furthermore, cell adhesion to .alpha.4.beta.1 **ligands** [VCAM-1 (vascular cell adhesion mol.-1), FN40 (40 kDa chymotryptic fragment of

fibronectin) and CS1] was greatly impaired in both RD and Jurkat cells, and cell spreading on immobilized FN40 protein was almost completely eliminated. Thus we conclude that intracellular **single-chain antibodies** may be used to reduce or eliminate cell-surface expression of a specific integrin, with specific functional consequences. This approach should be generally applicable to other integrin subunits.

- TI Intracellular **single-chain antibody** inhibits integrin VLA-4 maturation and function
- SO Biochem. J. (1996), 318(2), 591-596
CODEN: BIJOAK; ISSN: 0264-6021
- AB A **single-chain antibody** construct was prepd. contg. the VH and VL regions of anti-(integrin .alpha.4) antibody HP1/2, an interchain **linker** and a KDEL endoplasmic reticulum retention sequence. Intracellular expression of this **single-chain antibody** caused cell-surface expression of .alpha.4.beta.1 integrin to be decreased by 80% on selected RD cells and by 65-100% on selected Jurkat cells, relative to mock transfectants. Immunopptn. from **single-chain-antibody-transfected** cells showed that the **single-chain antibody** was complexed with the integrin .alpha.4 and .beta.1 subunits, and the diminished sizes of .alpha.4 and .beta.1 were consistent with impaired maturation. Furthermore, cell adhesion to .alpha.4.beta.1 **ligands** [VCAM-1 (vascular cell adhesion mol.-1), FN40 (40 kDa chymotryptic fragment of fibronectin) and CS1] was greatly impaired in both RD and Jurkat cells, and cell spreading on immobilized FN40 protein was almost completely eliminated. Thus we conclude that intracellular **single-chain antibodies** may be used to reduce or eliminate cell-surface expression of a specific integrin, with specific functional consequences. This approach should be generally applicable to other integrin subunits.
- IT Animal cell line
(RD; intracellular **single-chain antibody** inhibits integrin VLA-4 maturation and function)
- IT Transformation, genetic
(intracellular **single-chain antibody** inhibits integrin VLA-4 maturation and function)
- IT **Antibodies**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(intracellular **single-chain antibody** inhibits integrin VLA-4 maturation and function)
- IT Animal cell line
(JURKAT, intracellular **single-chain antibody** inhibits integrin VLA-4 maturation and function)
- IT Integrins
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.alpha.4.beta.1, intracellular **single-chain antibody** inhibits integrin VLA-4 maturation and function)

L10 ANSWER 5 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:426438 HCAPLUS

DOCUMENT NUMBER: 125:112531

TITLE: A single-chain Fv reactive with the Goodpasture antigen

AUTHOR(S): Ross, Galum N.; Turner, Neil; Savage, Philip; Cashman,

Stephen J.; Spooner, Robert A.; Pusey, Charles D.

CORPORATE SOURCE: Royal Postgraduate Medical School (CNR, NT, SJC, CDP),

SOURCE: Hammersmith Hospital, London, UK
Lab. Invest. (1996), 74(6), 1051-1059
CODEN: LAINAW; ISSN: 0023-6837
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Goodpasture's disease is defined by the presence of autoantibodies to the glomerular basement membrane and characterized clin. by rapidly progressive glomerulonephritis and pulmonary hemorrhage. P1, a murine monoclonal antibody to the Goodpasture antigen [the noncollagenous domain of the .alpha.3 chain of type IV collagen, .alpha.3(IV)NC1], has been a valuable reagent in investigating the pathogenesis of this disorder. The purpose here was to generate and characterize a recombinant form of P1 as a single-chain Fv (scFv). First strand cDNA was made from RNA extd. from the P1 hybridoma cell line, and DNA encoding the antibody light and heavy chain variable domains was amplified by polymerase chain reaction, using universal oligonucleotides. The purified products were ligated sequentially into an expression plasmid sepd. by a sequence encoding a 15 amino acid flexible oligopeptide **linker**. The resulting scFv was expressed in E. coli. Functional scFv, designated HBR-3, was obtained by denaturing and refolding the expressed product. HBR-3 was shown by

ELISA, immunoblotting, and immunohistol. techniques, to have the same specificity for .alpha.3(IV)NC1 as P1 and autoantibodies from patients with Goodpasture's disease. HBR-3 and P1 were shown to have similar affinity for their mutual **ligand**. On sections of normal human kidney, the scFv bound only to glomerular basement membrane and distal tubular basement membrane. It did not bind to the glomerular basement membrane

of patients with Alport's syndrome, in whom the Goodpasture antigen is often not expressed in an antigenic form. The authors have, therefore, generated a scFv which reproduces the specific binding properties of the parent monoclonal antibody, P1. The potential of HBR-3 as a diagnostic reagent in Alport's syndrome has been demonstrated. The development of this recombinant mol. should permit new approaches to the investigation

of Goodpasture's disease.

SO Lab. Invest. (1996), 74(6), 1051-1059
CODEN: LAINAW; ISSN: 0023-6837

AB Goodpasture's disease is defined by the presence of autoantibodies to the glomerular basement membrane and characterized clin. by rapidly progressive glomerulonephritis and pulmonary hemorrhage. P1, a murine monoclonal antibody to the Goodpasture antigen [the noncollagenous domain of the .alpha.3 chain of type IV collagen, .alpha.3(IV)NC1], has been a valuable reagent in investigating the pathogenesis of this disorder. The purpose here was to generate and characterize a recombinant form of P1 as a single-chain Fv (scFv). First strand cDNA was made from RNA extd. from the P1 hybridoma cell line, and DNA encoding the antibody light and heavy chain variable domains was amplified by polymerase chain reaction, using universal oligonucleotides. The purified products were ligated sequentially into an expression plasmid sepd. by a sequence encoding a 15 amino acid flexible oligopeptide **linker**. The resulting scFv was expressed in E. coli. Functional scFv, designated HBR-3, was obtained by denaturing and refolding the expressed product. HBR-3 was shown by

ELISA, immunoblotting, and immunohistol. techniques, to have the same specificity for .alpha.3(IV)NC1 as P1 and autoantibodies from patients with Goodpasture's disease. HBR-3 and P1 were shown to have similar affinity for their mutual **ligand**. On sections of normal human kidney,

the scFv bound only to glomerular basement membrane and distal tubular basement membrane. It did not bind to the glomerular basement membrane of patients with Alport's syndrome, in whom the Goodpasture antigen is often not expressed in an antigenic form. The authors have, therefore, generated a scFv which reproduces the specific binding properties of the parent monoclonal antibody, P1. The potential of HBR-3 as a diagnostic reagent in Alport's syndrome has been demonstrated. The development of this recombinant mol. should permit new approaches to the investigation of Goodpasture's disease.

IT **Antibodies**

RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(monoclonal, **single-chain** Fv antibody reactive with Goodpasture antigen in relation to diagnosis of Alport's syndrome)

L10 ANSWER 6 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:480617 HCAPLUS

DOCUMENT NUMBER: 121:80617

TITLE: Structure of a **single-chain antibody** variable domain (Fv) fragment complexed with a carbohydrate antigen at 1.7-Å resolution

AUTHOR(S): Zdanov, Alexander; Li, Yunge; Bundle, David R.; Deng, Su-Jun; MacKenzie, C. Roger; Narang, Saran A.; Young, N. Martin; Cygler, Mirosław

CORPORATE SOURCE: Bio. Res. Inst., Natl. Res. Counc. Canada, Montreal, PQ, H4P 2R2, Can.

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1994), 91(14), 6423-7

CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors describe here the 1.7-Å resolution structure of a **single-chain antibody** variable domain (scFv) mol., based on the carbohydrate-binding antibody Se155-4, complexed with the trisaccharide **ligand** .alpha.-D-Gal(1.fwdarw.2)[.alpha.-D-Abe(1.fwdarw.3)].alpha.-D-Manp1.fwdarw.OMe, where Abe is abequose. The scFv expressed in Escherichia coli has the variable region light chain to heavy chain polarity with the domains connected by a 19-residue **linker**. Although the **linker** is partially disordered in the crystal, the packing of the mols. suggests a monomeric state of the scF. The carbohydrate adopts a different conformation about the Man-Gal linkage than was obsd. previously in the Fab-trisaccharide complex. Instead of a direct hydrogen bond between O2Abe and O2Gal, these two

atoms are bridged by a water mol. in the present complex.

TI Structure of a **single-chain antibody** variable domain (Fv) fragment complexed with a carbohydrate antigen at 1.7-Å resolution

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atoms

are bridged by a water mol. in the present complex.

L10 ANSWER 7 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:296286 HCAPLUS

DOCUMENT NUMBER: 120:296286

TITLE: **Ligand Binding to Anti-Fluorescyl Antibodies: Stability of the Antigen Binding Site**

AUTHOR(S): Mueller, J. D.; Nienhaus, G. U.; Tetin, S. Y.; Voss, E. W.

CORPORATE SOURCE: Department of Physics, University of Illinois, Urbana,

IL, 61801-3080, USA

SOURCE: Biochemistry (1994), 33(20), 6221-7

CODEN: BICHAW; ISSN: 0006-2960

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The problem of protein stability is addressed with spectroscopic studies of equil. and kinetic properties of the binding of fluorescein to high-affinity monoclonal anti-fluorescyl **antibodies** (Mab 4-4-20), Fab fragments, and **single-chain antibodies** (SCA). SCA mols. contain only the variable domains of the antibody and an amino acid **linker**. The influence of glycerol on the antigen binding reaction is studied by CD, fluorescence, and absorption spectroscopy. The presence of glycerol in the solvent lowers the affinity of SCA for the **ligand** drastically, and the affinity even decreases toward lower temps. This effect is not obsd. in Fab and Mab. Anal. of the temp. jump kinetics shows that the dissocn. reaction can be modeled as a two-state transition. The CD spectra indicate that the domain structure of the SCA remains unaltered in the presence of glycerol. Therefore, it is concluded that glycerol promotes the dissocn. of the two variable domains of SCA. In Fab and Mab, the const. domains provide addnl. stabilization of the mol. structure at the antigen binding site.

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IT Force
(hydrophobic, in monoclonal antibody assocn. with fluorescein
ligand)
IT 56-81-5, Glycerol, uses
RL: USES (Uses)
(antibody-ligand complex stability response to)

L10 ANSWER 8 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:75073 HCAPLUS

DOCUMENT NUMBER: 120:75073

TITLE: Construction, expression, and activity of a bivalent
bispecific **single-chain
antibody**

AUTHOR(S): Mallender, William D.; Voss, Edward W., Jr.

CORPORATE SOURCE: Dep. Microbiol., Univ. Illinois, Urbana, IL, 61801,
USA

SOURCE: J. Biol. Chem. (1994), 269(1), 199-206

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB This report describes the design, construction, and expression of a
bivalent bispecific **single-chain antibody**
(SCA) protein in Escherichia coli. The bispecificity of the bivalent
protein was based on two previously constructed monovalent **single
-chain antibody** mols. possessing distinct
specificities, SCA-4-4-20 (anti-fluorescein) and SCA 04-01
(anti-single-stranded DNA). A flexible **linker**, modeled after a
secreted fungal cellulase protein, was incorporated as the interdomain
linker covalently joining the two active sites. Bivalent
bispecific SCA protein that accumulated in bacteria as insol. inclusion
bodies was harvested, denatured, refolded, and affinity-purified in
vitro.

Affinity-purified bivalent bispecific SCA showed nearly identical
ligand binding properties at each site relative to the individual
monovalent **single-chain antibody** prototype
mols. In both solid and soln. phase binding assays, the bivalent
bispecific **single-chain antibody**
simultaneously bound both **ligands** (fluorescein and (dT)6).
Construction of a model bivalent bispecific mol. provides a foundation

for
future assembly of similar mols. designed to identify parameters involved
in enhanced binding of **antibodies** due to avidity and dual
specificity.

TI Construction, expression, and activity of a bivalent bispecific
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ligand binding properties at each site relative to the individual monovalent **single-chain antibody** prototype mols. In both solid and soln. phase binding assays, the bivalent bispecific **single-chain antibody** simultaneously bound both **ligands** (fluorescein and (dT)6). Construction of a model bivalent bispecific mol. provides a foundation for future assembly of similar mols. designed to identify parameters involved in enhanced binding of **antibodies** due to avidity and dual specificity.

ST bispecific **single chain antibody**
 IT Immunoassay
 (DAB (dual antigen-binding), bivalent bispecific **single-chain antibody** for)
 IT **Antibodies**
 RL: PREP (Preparation)
 (bispecific **single-chain**, prepn. and characterization of)
 IT Molecular association
 (of antigens with bispecific **single-chain antibody**)
 IT Deoxyribonucleic acids
 RL: PREP (Preparation)
 (single-stranded, bispecific **single-chain antibody** to fluorescein and, prepn. and characterization of)
 IT 150883-28-6
 RL: PRP (Properties)
 (as interdomain **linker** peptide for bispecific **single-chain antibody**)
 IT 2321-07-5P, Fluorescein
 RL: PREP (Preparation)
 (bispecific **single-chain antibody** to single-stranded DNA and, prepn. and characterization of)

L10 ANSWER 9 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:52105 HCAPLUS

DOCUMENT NUMBER: 120:52105

TITLE: Crystallization of single-chain Fv proteins

AUTHOR(S): Essig, Nina Z.; Wood, James F.; Howard, Andrew J.; Raag, Reetta; Whitlow, Marc

CORPORATE SOURCE: Protein Eng. Dep., Enzon Inc., Piscataway, NJ, 08854-3998, USA

SOURCE: J. Mol. Biol. (1993), 234(3), 897-901

CODEN: JMOBAK; ISSN: 0022-2836

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Single-chain Fv (sFv) proteins consist of the variable heavy chain (VH) and variable light chain (VL) domains of an antibody, covalently joined

by an engineered polypeptide **linker**. The authors report the crystn. of single-chain Fv's with specificities for fluorescein (4-4-20 sFv) and the TAG-72 pancreatic carcinoma glycoprotein antigen (CC49 sFv). Concn. of these proteins, preliminary to crystn., resulted in a monomer-multimer equil., causing aggregation which interferes with crystn. Aggregation

has been obsd. to depend primarily on an intact **linker** between VL and VH domains, although other factors are likely to modulate this phenomenon as well, including the specific identity of Fv and **ligand**, presence or absence of the **ligand**, **linker** length and possibly sequence. The authors have found two

methods to overcome sFv aggregation, both of which yield x-ray diffraction quality crystals. The first, discovered serendipitously, is by introducing a proteolytic clip into the **linker** region (effectively yielding an Fv fragment). The second is the purifn. of the sFv dimer form, with **linker** regions intact, from an equil. mixt. of aggregates. The sFv mol. assocn. in a dimer is believed to be unusual in that each VL/VH interface may not be formed by the two **linker**-connected VL and VH domains, but rather by interaction of VL and VH domains from two distinct sFv monomers. Structure detn. of the CC49 sFv dimer, with the 14-residue **linker** designated 212, is underway to test this model. Increasing **linker** length, to relieve steric strain on the monomer, and inclusion of the appropriate antigen, to slow transitions between monomeric and multimeric forms, may prove valuable strategies with sFv proteins less amenable to crystn.

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ST **single chain antibody** Fv crystn
IT Crystallization
(of **single-chain antibody** Fv fragments)

IT Immunoassay
(size exclusion high-performance chromatog., for **single-chain antibody** Fv fragment prepn.)

IT Antigens
RL: BIOL (Biological study)
(TAG-72 (tumor-assocd. glycoprotein 72), **single-chain antibody** Fv fragments to, crystn. of)

IT **Antibodies**
RL: BIOL (Biological study)
(monoclonal, **single-chain** Fv, crystn. of)

IT Molecular association

(self-, of **single-chain antibody** Fv fragments, crystn. in relation to)

IT 130838-28-7
 RL: USES (Uses)
 (as **linker** for **single-chain antibody** Fv fragments, crystn. in relation to)

IT 9014-01-1, Subtilisin
 RL: USES (Uses)
 (in **single-chain antibody** Fv fragment prepn.)

IT 2321-07-5, Fluorescein
 RL: USES (Uses)
 (**single-chain antibody** Fv fragments to, crystn. of)

L10 ANSWER 10 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:469926 HCAPLUS

DOCUMENT NUMBER: 119:69926

TITLE: Construction, characterization, and selected site-specific mutagenesis of an anti-single-stranded DNA single-chain autoantibody

AUTHOR(S): Rumbley, Catherine A.; Denzin, Lisa K.; Yantz, Leslie;

Tetin, Sergey Yu.; Voss, Edward W., Jr.
 CORPORATE SOURCE: Dep. Microbiol., Univ. Illinois, Urbana, IL, 61801, USA

SOURCE: J. Biol. Chem. (1993), 268(18), 13667-74
 CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Single-chain antibodies** are comprised of Ig light and heavy chain variable domains joined through a polypeptide **linker**. A **single-chain** autoantibody, contg. the 14-amino acid 212-polypeptide **linker** (GSTSGSGKSSEGKG), was constructed based on the light and heavy chain variable region gene sequences of anti-single-stranded DNA autoantibody BV04-01 (IgG2b,.kappa.). Following protein expression in Escherichia coli, denaturation, refolding, and affinity purifn., **single-chain** autoantibody 04-01 binding with single-stranded DNA and poly(dT) was characterized in solid-phase and soln.-phase assays. Homopolymer **ligand** binding results demonstrated that **single-chain** autoantibody 04-01 possessed anti-DNA binding properties similar to BV04-01 IgG and Fab fragments. Based on x-ray crystallog. analyses of BV04-01, site-specific mutagenesis studies were conducted on 2 residues (L32Tyr and H100aTrp) involved in arom. stacking interactions with the middle thymidine of a (dT)3 **ligand**

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IT **Antibodies**

RL: PREP (Preparation)

(auto-, to single-stranded DNA, prepn. and characterization of **single-chain**)

L10 ANSWER 11 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:145375 HCAPLUS

DOCUMENT NUMBER: 118:145375

TITLE: Efficient production of a functional **single-chain** antidigoxin antibody via an engineered Bacillus subtilis expression-secretion system

AUTHOR(S): Wu, Xu Chu; Ng, Shi Chung; Near, Richard I.; Wong, Sui

CORPORATE SOURCE: Lam
Dep. Biol. Sci., Univ. Calgary, Calgary, AB, T2N 1N4, Can.

SOURCE: Bio/Technology (1993), 11(1), 71-6
CODEN: BTCHDA; ISSN: 0733-222X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A B. subtilis expression-secretion system was applied to produce a functional antidigoxin SCA (**single-chain antibody** consisting of VL-linker-VH) and the individual variable domains of light (VL) and heavy (VH) chains. The secreted antidigoxin SCA can be affinity purified in one step by applying the culture supernatant directly to a ouabain-Sepharose column. N-terminal sequence detn. indicated that the protein has the expected N-terminus

with the signal peptide properly processed. Affinity and **ligand** specificity studies demonstrated that the engineered antidigoxin SCA has almost identical properties as those of the parental monoclonal antibody. The use of B. subtilis WB600, an engineered, 6-extracellular protease-deficient strain, is vital for the prodn. of antidigoxin SCA in high quality and quantity (5 mg/L in a shake flask culture). All the secreted SCAs are biol. active. The ability to produce secreted SCAs by the B. subtilis expression system provides a simple and efficient means

to analyze the binding properties of engineered **antibodies** generated through rational design or site-directed mutagenesis.

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ST digoxin **single chain antibody** Bacillus vector

IT **Antibodies**
RL: BIOL (Biological study)
(to digoxin, **single-chain** recombinant, Bacillus subtilis expression-secretion system for)

L10 ANSWER 12 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1992:233469 HCAPLUS

DOCUMENT NUMBER: 116:233469

TITLE: Characterization of interactions involving anti-metatype antibodies and immune complexes

AUTHOR(S): Weidner, Karla M.; Voss, Edward W., Jr.

CORPORATE SOURCE: Dep. Microbiol., Univ. Illinois, Urbana, IL, 61801, USA

SOURCE: Mol. Immunol. (1992), 29(3), 303-12

CODEN: MOIMD5; ISSN: 0161-5890

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Immunization with high affinity anti-fluorescein monoclonal antibody 4-4-20 affinity labeled with fluorescein 5-isothiocyanate into a rabbit elicited **antibodies** specific for the liganded conformation of 4-4-20 (termed anti-metatype **antibodies**). Reaction of liganded 4-4-20 with anti-metatype **antibodies** caused a delay (up to 23-fold) in the rate of dissocn. of the fluorescein **ligand** from the active site. In this study, structural analogs of fluorescein, including fluorescein 5-isothiocyanate, fluorescein 6-isothiocyanate, 5-dichlorotriazinyl aminofluorescein, and 5-carboxyfluorescein, were

bound

by monoclonal antibody 4-4-20 and anti-metatype antibody reactivity was obsd. through delay in the dissocn. rate of **ligand** from Mab 4-4-20. Significant delays (ranging from 5-242-fold) were obsd. for all structural analogs examd. indicating that 4-4-20 maintained similar but not necessarily identical conformations upon binding fluorescein structural analogs. Addnl., fluorescein 5-isothiocyanate and fluorescein 6-isothiocyanate were **conjugated** to carrier mols. of increasing mol. wt (ranging from 225 to 14,600 daltons) in an attempt to sterically interfere with metatopes at the mouth of the active site and localize regions of anti-metatype antibody binding. These fluorescein-**conjugated** compds. were reacted with 4-4-20, and binding of anti-metatype **antibodies** delayed dissocn. rates from 24- to >1500-fold. Thus, the mechanism whereby anti-metatype **antibodies** delay the release of fluorescein **ligands** from the active site probably does not solely involve steric hindrance of the **ligand** due to binding of anti-metatype **antibodies** at the mouth of the active site. Studies with 4-4-20 Fab fragments and a **single chain** deriv. of 4-4-20 (consisting of the variable regions tethered by a 14 amino acid **linker**) indicated that anti-metatype reactivity was specific for the Ig variable region.

SO Mol. Immunol. (1992), 29(3), 303-12

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L10 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1991:605545 HCAPLUS

DOCUMENT NUMBER: 115:205545

TITLE: Conformational stability, folding, and **ligand**-binding affinity of single-chain Fv immunoglobulin fragments expressed in Escherichia coli

AUTHOR(S): Pantoliano, Michael W.; Bird, Robert E.; Johnson, Syd;

Asel, Eric D.; Dodd, Steven W.; Wood, Jay F.; Hardman, Karl D.

CORPORATE SOURCE: Genex Corp., Gaithersburg, MD, 20872, USA

SOURCE: Biochemistry (1991), 30(42), 10117-25

CODEN: BICHAW; ISSN: 0006-2960

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A fluorescein-binding single-chain Fv (scFv) was chosen as a model for the

study of the physicochem. parameters assocd. with synthetic IgG fragments.

Three such scFv proteins were designed from the primary sequences of one anti-fluorescein monoclonal antibody (Mab 4.4.20). These were constructed with varying-length interdomain peptide **linkers** of between 12 and 25 residues, expressed in E. coli, and the protein folding, stability, and antigen-binding characteristics were assessed. Efficient renaturation

could be accomplished in vitro to yield approx. 26 mg of active scFv/L of fermn. Scatchard anal. for fluorescein **ligand** binding revealed that the scFv designs come within 2-fold of the $K_a = 1.99 \times 10^9$ obsd. for the parental 4.4.20 Fab and have identical stoichiometries. Reversible solvent denaturation studies demonstrated that the

unfolding/refolding equil. for the scFv proteins can be fit to a simple 2-state model and that two of the scFv designs were found to be slightly more stable than single IgG domains (VL and CL) when assessed in terms of the free energy of unfolding, $\Delta G_{\text{unfolding}}$, or nearly identical to other multiple domain Ig proteins such as light chains and Fab's when relative transition midpoints, C_m , are compared. **Linkers** which conferred conformational flexibility beyond the minimally required length of 12 residues were found to have a stabilizing effect. By these

criteria
of **ligand**-binding function and protein stability, the scFv proteins were found to be bona fide minimal replicas of their parental

IgG mols.

TI Conformational stability, folding, and **ligand**-binding affinity of single-chain Fv immunoglobulin fragments expressed in Escherichia coli

SO Biochemistry (1991), 30(42), 10117-25
CODEN: BICHAW; ISSN: 0006-2960

AB A fluorescein-binding single-chain Fv (scFv) was chosen as a model for the study of the physicochem. parameters assocd. with synthetic IgG fragments.

Three such scFv proteins were designed from the primary sequences of one anti-fluorescyl monoclonal antibody (Mab 4.4.20). These were constructed with varying-length interdomain peptide **linkers** of between 12 and 25 residues, expressed in E. coli, and the protein folding, stability, and antigen-binding characteristics were assessed. Efficient renaturation could be accomplished in vitro to yield approx. 26 mg of active scFv/L of fermn. Scatchard anal. for fluorescein **ligand** binding revealed that the scFv designs come within 2-fold of the $K_a = 1.99 \times 10^9$ obsd. for the parental 4.4.20 Fab and have identical stoichiometries. Reversible solvent denaturation studies demonstrated that the unfolding/refolding equil. for the scFv proteins can be fit to a simple 2-state model and that two of the scFv designs were found to be slightly more stable than single IgG domains (VL and CL) when assessed in terms of the free energy of unfolding, $\Delta G_{\text{unfolding}}$, or nearly identical to other multiple domain Ig proteins such as light chains and Fab's when relative transition midpoints, C_m , are compared. **Linkers** which conferred conformational flexibility beyond the minimally required length of 12 residues were found to have a stabilizing effect. By these

criteria
of **ligand**-binding function and protein stability, the scFv proteins were found to be bona fide minimal replicas of their parental

IgG mols.

IT **Ligands**
RL: BIOL (Biological study)
(Ig single-chain Fv fragment binding to, peptide **linker** in relation to)

IT Molecular association
(of Ig single-chain Fv fragment with **ligand**, properties of, peptide **linker** in relation to)

IT Conformation and Conformers
(of Ig single-chain Fv fragment, stability of, peptide **linker** in relation to)

IT Free energy
(of unfolding, of Ig single-chain Fv fragment, peptide **linker** in relation to)

IT Immunoglobulins

RL: BIOL (Biological study)
(single-chain recombinant Fv fragment of, folding and stability and
antigen-binding properties of, peptide **linker** in relation to)

IT **Antibodies**
RL: BIOL (Biological study)
(monoclonal, **single-chain** recombinant Fv fragment
of, folding and stability and antigen-binding properties of, peptide
linker in relation to)

IT 130838-28-7 136476-16-9 136476-17-0
RL: BIOL (Biological study)
(as **linker** in recombinant single-chain Fv Ig fragment,
fragment physicochem. parameters in relation to)

IT 2321-07-5, Fluorescein
RL: BIOL (Biological study)
(monoclonal antibody to, single-chain recombinant Fv fragment of,
folding and stability and antigen-binding properties of, peptide
linker in relation to)

L10 ANSWER 14 OF 15 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:533677 HCAPLUS
DOCUMENT NUMBER: 115:133677
TITLE: Single-chain site-specific mutations of
fluorescein-amino acid contact residues in high
affinity monoclonal antibody 4-4-20
AUTHOR(S): Denzin, Lisa K.; Whitlow, Marc; Voss, Edward W., Jr.
CORPORATE SOURCE: Dep. Microbiol., Univ. Illinois, Urbana, IL, 61801,
USA
SOURCE: J. Biol. Chem. (1991), 266(21), 14095-103
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Previous crystallog. studies of high affinity anti-fluorescein monoclonal
antibody 4-4-20 ($K_a = 1.7 \times 10^{10} \text{ M}^{-1}$) complexed with fluorescein
ligand resolved active site contact residues involved in binding.
For better definition of the relative roles of 3 light chain antigen
contact residues (L27dhis, L32tyr and L34arg), 4 site-specific mutations
(L27dhis to L27dlys, L32tyr to L32phe, and L34arg and L34his) were
generated and expressed in single-chain antigen binding derivs. of
monoclonal antibody 4-4-20 contg. 2 different polypeptide **linkers**
(SCA 4-4-20/205c, 25 amino acids and SCA 4-4-20/212, 14 amino acids).
L27dhis and L32tyr were necessary for wild type binding affinities;
however, they were not required for near-wild type Q_{max} values (where
 Q_{max} is the max. fluorescein fluorescence quenching expressed as percent).
Tyrosine L32 which hydrogen bonds with **ligand** was also
characterized at the haptenic level through the use of
9-hydroxyphenylfluorone which lacks the carboxyl group to which L32
tyrosine forms a hydrogen bond. Wild type SCA and mutant L32phe
possessed
similar HPF binding characteristics. Active site contact residue L34arg
was important for fluorescein quenching max. and binding affinity (L34his
mutant); however, substitution of lysine for arginine at L34 did not have
a significant effect on obsd. Q_{max} value. In addn., substitutions had no
effect on structural and topol. characteristics, since all mutants
retained similar idiotypic and metatypic properties. Finally, 2
linkers were comparatively examd. to det. relative contributions
to mutant binding properties and stability. No **linker** effects
were obsd. Thus, these light chain fluorescein contact residues are
important in the binding pocket of monoclonal antibody 4-4-20.

SO J. Biol. Chem. (1991), 266(21), 14095-103

CODEN: JBCHA3; ISSN: 0021-9258

AB Previous crystallog. studies of high affinity anti-fluorescein monoclonal antibody 4-4-20 ($K_a = 1.7 \times 10^{10} \text{ M}^{-1}$) complexed with fluorescein **ligand** resolved active site contact residues involved in binding. For better definition of the relative roles of 3 light chain antigen contact residues (L27dhis, L32tyr and L34arg), 4 site-specific mutations (L27dhis to L27dlys, L32tyr to L32phe, and L34arg and L34his) were generated and expressed in single-chain antigen binding derivs. of monoclonal antibody 4-4-20 contg. 2 different polypeptide **linkers** (SCA 4-4-20/205c, 25 amino acids and SCA 4-4-20/212, 14 amino acids). L27dhis and L32tyr were necessary for wild type binding affinities; however, they were not required for near-wild type Q_{max} values (where

Q_{max}

is the max. fluorescein fluorescence quenching expressed as percent). Tyrosine L32 which hydrogen bonds with **ligand** was also characterized at the haptenic level through the use of 9-hydroxyphenylfluorone which lacks the carboxyl group to which L32 tyrosine forms a hydrogen bond. Wild type SCA and mutant L32phe

possessed

similar HPF binding characteristics. Active site contact residue L34arg was important for fluorescein quenching max. and binding affinity (L34his mutant); however, substitution of lysine for arginine at L34 did not have a significant effect on obsd. Q_{max} value. In addn., substitutions had no effect on structural and topol. characteristics, since all mutants retained similar idiotypic and metatypic properties. Finally, 2 **linkers** were comparatively examd. to det. relative contributions to mutant binding properties and stability. No **linker** effects were obsd. Thus, these light chain fluorescein contact residues are important in the binding pocket of monoclonal antibody 4-4-20.

ST fluorescein monoclonal antibody **ligand** binding mutation

IT **Antibodies**

RL: BIOL (Biological study)
(monoclonal, to fluorescein, **ligand** binding by, amino acid contact residues in, **single-chain** site-specific mutations in study of)

IT Mutation

(site-specific, in **ligand**-binding pocket, of monoclonal antibody to fluorescein, in contact residue role study)

IT 2321-07-5, Fluorescein

RL: BIOL (Biological study)
(monoclonal antibody to, **ligand** binding by, amino acid contact residues in, single-chain site-specific mutations in study of)

IT 60-18-4, Tyrosine, biological studies 71-00-1, Histidine, biological studies 74-79-3, Arginine, biological studies

RL: BIOL (Biological study)
(of **ligand**-binding pocket, of monoclonal antibody to fluorescein, mutation in, in contact residue role study)

L10 ANSWER 15 OF 15 HCAPLUS : COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1991:4559 HCAPLUS

DOCUMENT NUMBER: 114:4559

TITLE: Immunological and structural characterization of a high affinity anti-fluorescein **single-chain antibody**

AUTHOR(S): Bedzyk, William D.; Weidner, Karla M.; Denzin, Lisa K.; Johnson, Leslie S.; Hardman, Karl D.; Pantoliano, Michael W.; Asel, Eric D.; Voss, Edward W., Jr.

CORPORATE SOURCE: Dep. Microbiol., Univ. Illinois, Urbana, IL, 61801, USA

SOURCE: J. Biol. Chem. (1990), 265(30), 18615-20

DOCUMENT TYPE:

Journal

LANGUAGE:

English

- AB **Single-chain antibody** of the (NH₂) VL-linker-VH (COOH) design, was constructed based on prototype high affinity anti-fluorescein monoclonal antibody (mAb) 4-4-20. Purified **single-chain antibody** (SCA) 4-4-20/212 was studied relative to Ig mAb 4-4-20 in terms of **ligand** binding, kinetics, idiotype, metatype, and stability in denaturing agents. **Ligand**-binding data correlated with metatypic relatedness of the liganded site. Anti-metatypic reagents reacted preferentially with the liganded conformer of the 4-4-20 antibody active site and were unreactive with free **ligand** and the non-liganded (idiotypic) state. All results were consistent with the conclusion that SCA 4-4-20/212, with a 14-amino acid **linker**, folded into a native conformational state that closely simulated the prototypical mAb. GndHCl unfolding and refolding studies demonstrated H and L chain variable domain intrinsic stability between SCA 4-4-20/212 and a 50 kDa antigen-binding fragment were nearly identical. This suggested CH1 and CL domain interactions may be more prevalent in V region mol. dynamics than in structure.
- TI Immunological and structural characterization of a high affinity anti-fluorescein **single-chain antibody**
- SO J. Biol. Chem. (1990), 265(30), 18615-20
CODEN: JBCHA3; ISSN: 0021-9258
- AB **Single-chain antibody** of the (NH₂) VL-linker-VH (COOH) design, was constructed based on prototype high affinity anti-fluorescein monoclonal antibody (mAb) 4-4-20. Purified **single-chain antibody** (SCA) 4-4-20/212 was studied relative to Ig mAb 4-4-20 in terms of **ligand** binding, kinetics, idiotype, metatype, and stability in denaturing agents. **Ligand**-binding data correlated with metatypic relatedness of the liganded site. Anti-metatypic reagents reacted preferentially with the liganded conformer of the 4-4-20 antibody active site and were unreactive with free **ligand** and the non-liganded (idiotypic) state. All results were consistent with the conclusion that SCA 4-4-20/212, with a 14-amino acid **linker**, folded into a native conformational state that closely simulated the prototypical mAb. GndHCl unfolding and refolding studies demonstrated H and L chain variable domain intrinsic stability between SCA 4-4-20/212 and a 50 kDa antigen-binding fragment were nearly identical. This suggested CH1 and CL domain interactions may be more prevalent in V region mol. dynamics than in structure.
- ST anti fluorescein **single chain antibody**
- IT Conformation and Conformers
(of anti-fluorescein high-affinity recombinant **single-chain antibody**)
- IT **Antibodies**
RL: BIOL (Biological study)
(to fluorescein, **single-chain** recombinant high-affinity, characterization of)
- IT 130838-28-7
RL: BIOL (Biological study)
(as **linker** in high-affinity anti-fluorescein **single-chain antibody**)